STUDIES ON DIATOMS ALONG THE SOUTH WEST COAST OF INDIA IN RELATION TO THE HYDROLOGICAL PARAMETERS

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BY

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Dedicated to my parents, husband and children

DECLARATION

I hereby declare that the thesis entitled **"Studies on diatoms in relation to the hydrological parameters of the south west coast of India"** is an authentic record of research work carried out by me under the guidance and supervision of Dr.C.P. Gopinathan, Principal Scientist, Central Marine Fisheries Research Institute, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Botany of the Cochin University of Science and Technology and no part there of has been previously formed the basis of the award of any degree, diploma or associateship in any University.

Bindhu

Date : 13-07-2006 .

(BINDHU. K. B.)

CERTIFICATE

This is to certify that the thesis entitled **"Studies on diatoms along the south west coast of India in relation to the hydrological parameters"** is an authentic record of research work carried out by Bindhu. K.B. (Reg.No. 2235) under my guidance and supervision in Central Marine Fisheries Research Institute, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Botany of the Cochin University of Science and Technology, and no part thereof has been previously formed the basis of the award of any degree, diploma and associateship in any University.

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Preface

The process of primary production over much of the aquatic system of the earth is carried out by the Phytoplankton, "the grass" of the surface of the rivers, lakes and oceans. Majority of the phytoplankton are micro algae and belong to diverse group of premature, non flowering plants. In the absence of compatible morphological mechanisms, these organisms especially the unicellular forms, possess rather flexible physiological characteristics as an adaptation to the vagaries of the aquatic ecosystem. This property raises them to the status of 'good model' organisms to be used to answer most of the questions of the biological significance. The small, simple, single-celled construction of most of the phytoplankton along with their rapid growth rate further establish the relevance of these organisms in various physiological and ecological studies.

The species composition of the phytoplankters in the aquatic environment is greatly influenced by the biological, physical and chemical factors operating directly or indirectly in the habitat. The diatoms are most common in both fresh water and marine habitats among the phytoplankton. Diatoms constitute 80-90% of the phytoplankton and form important component in the food web of

the shoal fishes. Thus the diatoms, which occupies the vital role as primary producers in marine ecosystem performs an equally important role outside their natural habitats like in the hatchery system also.

The ecosystem in which diatoms are present is highly dynamic since they are constantly exposed to the several fluctuating environmental conditions and seasonal variations. The importance of the study of diatoms was recognized at least a century ago and has been the subject of intensive study in all the maritime countries of the world. In European countries there are considerable works on diatoms hence; literature dealing with it is vast and varied. But the study of diatoms in our country is still in infancy.

At present attempts are going on to maximize the production of the single cell protein by mass culturing of diatoms for feeding the larvae of crustaceans, molluscs and fin fishes in hatcheries and has been found to be a unique factor in determining the success of these enterprises.

Hence a study of diatoms with special reference to their hydrographic parameters is an essential prerequisite for any attempt of identification of fishery at species level and also to develop a hatchery feed. The present study was undertaken to estimate the

distribution of the diatoms along the south west coast of India with special reference to the hydrological factors. All the hydrographic factors affecting their distribution and abundance were studied including quantitative and qualitative parameters in the natural environment. However, an attempt has been made in the laboratory conditions to study the maximum exponential phase in culture medium by using the centric diatom *Cheatoceros calcitrans* by providing various concentrations of Walne's culture medium.

Another study was also undertaken to access the effect of elimination of few trace elements such as zinc, copper, cobalt, molybdenum and vitamins like vitamins B_{12} and B_1 in the laboratory culture of the diatom *Chaetoceros calcitrans* which is an important live feed in the hatchery and to determine the optimum concentration of the Walne's medium for its growth, since this is used for rearing the larvae of the economically important marine organisms such as crustaceans, molluscs, fin fishes and sea cucumber.

In view of the great attention drawn by the biologists, nutritionists and technologists in utilization of algae as one of the possibilities to bridge the inadequacy and scarcity of proteins and their use as live feed in aquaculture, the present study on influence of the medium without trace elements such as copper, zinc,

molybdenum, magnesium and cobalt in the growth of *Chaetoceros* has undertaken.

It is also hoped that the results and conclusion drawn from these investigations will be useful in the development of mass culture of the diatom in hatchery system.

The details of the investigations are presented in 5 chapters. Chapter I embodies general introduction to the subject studied where details of resource potentials and review of the earlier works are discussed. Chapter II gives a detailed account of diatom distribution and ecology along the south west coast of India in relation to hydrological parameters. Chapter III deals with the experiments conducted for the determination of the efficiency or optimum nutrient concentration for the maximum production of *Chaetoceros* culture. Chapter IV embodies the effect of the trace elements vitamins in the growth and biochemical composition of *Chaetoceros calcitrans*. Chapter V deals with a correlation study of diatom population of the west coast of India with the pelagic fishery resources, based on the data obtained from Fisheries Resources Assessment Division of CMFRI. The material and methods and a brief introduction to the topic are included in the respective chapters. The summary and the literature cited in text are included in reference section.

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Chapter 1

General Introduction

Phytoplankton has been recognized as an extremely important source of food for larval forms of aquatic organisms, since they constitute the microscopic plant life of the sea. Important components of the phytoplankters are: Diatoms, Dinoflagellates, Blue green algae, phytoflagellates, Coccolithophores and Nannoplankters. They play an important role in the biosynthesis of organic matter (primary production) in the aquatic ecosystem which directly or indirectly serves all the aquatic life as the basic food. It supplies both energy and essential nutritional requirements such as protein, carbohydrate and lipids since the phytoplankton forms the meadows of the sea.

Diatoms constitute major part of the phytoplankton. Their importance lies in the fact that they are the photosynthesizing organisms and serve as a vital first link in the food chain, either directly or indirectly of almost every animal in the sea. It is true that at sometimes in the early stage of life cycle all fish, molluscs and crustaceans are diatom feeders, at least in part. Diatom cells are unique; having rigid silica impregnated cell wall (the frustule) consisting of two parts (valves) with one (epitheca) overlapping the other (hypotheca) in the girdle region like a box. Diatoms are

broadly classified into Centrals and Pennales, based on the shape, structure and locomotion.

Johnstone (1908) reported that 'The diatoms are above all the most important organisms in the area regarded from the point of view of their significance as the producers of organic substances. The diatoms are the 'pastures of the sea' and correspond to the 'grass of the fields' of the land.' Gran (1930) has the opinion that "These enormous quantities of diatoms, without doubt, are the most important food for the pelagic copepods add indirectly for the fish larvae which develop after the great spring spawning period". Phifer (1933) likewise reported, "Marine diatoms are the principal sources upon which the fauna of the oceans depends for the energy necessary for existence. Undoubtedly the shore micro algae produce organic material forming nutritive substance for bacteria, which in turn are probably consumed by small protozoa. However the diatoms are directly consumed and produce organic food in much larger quantities since the aerial extent of their distribution is many times greater than shore forms."

A plant cannot live with a deficiency while an excess is toxic. A remarkable advance in the application and appreciation of significant role played by the trace elements in the health and

productivity of plants were observed in the past four decades. Pioneer work on the nutritional significance of trace elements began in 1928 and flourished throughout the 30's and later years. The recent spectacular rise in the trace elements in marine products has followed after a few catastrophic episodes such as the Minamata, occurrence of *itai-itai* disease caused by mercury and cadmium poisoning respectively.

1.1 Review of the literature

Menon (1931) has referred a few forms of diatoms and Gopala Iyer and Sankara Menon (1936) has given a list of diatoms collected from Madras Coastand Venkataraman (1939) has given an account of common fresh water diatoms of South India. The only comprehensive work on Indian marine diatoms is that of Subrahmanian (1946) where in he has given a systematic account of 171 forms. Gonzalves (1947) has recorded 126 species of diatoms from Bombay harbor. Gonzalves and Gandhi (1952, 1953 and 1954), Krishnamoorthy (1954) and Gandhi (1955) have been reported on the fresh water diatoms collected from various part of India. Prasad (1954) has given a list of diatoms occurring in the plankton at Mandapam area, with special reference to seasonal

variations. An account of marine diatoms from Indian waters was also given by Nair (1959) and Gopinathan (1975).

Seasonal variation of phytoplankton and productivity in the surface zone and back waters at Cochin was given by Gopinathan (1972) and Selvaraj *et al.* (2003). Quantitative investigation of Phytoplankton was studied by Henson (1887) who was the first to report on the seasonal variations. The plankton expeditions (Henson, 1887; Lobman, 1920) demonstrated the greater density of Plankton population in coastal waters as compared with the open sea. This inference gave rise to two important problems mainly the reasons for the greater productivity, and the factors influencing the spring plankton outburst.

1.1.1 Phytoplankton and hydrography

The present study has been carried out with a view to study the diatoms of selected areas along the south west coast of India with special reference to the hydrographic factors. Literatures related to the environment in south west coast of India revealed that food requirement in relation to fisheries in this part of Indian seas are scanty.

Several studies on phytoplankton have been made in the coastal and offshore regions of Indian seas. Subrahmanyan (1959

a, b) measured the standing crop of phytoplankton and came to the conclusion that the production on the west coast of India is of a high order comparable to some of the most productive areas in the temperate regions. Prasad and Nair (1960 and 1963) made a study of the seasonal variation and magnitude of production in the Gulf of Mannar on the south east coast of India. The results of investigations carried out along the shelf regions of India and Lakshadweep sea were discussed in relation to the potential living resources by Nair *et al.* (1968) and Nair (1970, 1974). Radhakrishna (1969) made a study of primary productivity in the shelf waters of Alleppy on the south west coast of India during the post monsoon period and Shah (1973) presented the seasonal variation of phytoplankton pigments in the Lakshadweep sea of Cochin. Further, Quasim *et al.* (1978) have discussed the biological productivity of coastal waters of India upto 50m depth and stated that the larger phytoplankton organisms (macroplankton) contributed greater spatial variation in primary production than the smaller forms (nannoplankton) Radhakrishna *et al.* (1978 a) studied some qualitative aspects of phytoplankton productivity from the coastal areas of east coast including some stations in the Bay of Bengal. Further, Radhakrishna *et al.* (1978 b, c) studied the primary productivity, chlorophyll *a* and related

parameters from the shelf and oceanic regions in North eastern Arabian Sea and Northern Arabian Sea.

Studies on the biology and ecology of the phytoplankton of various estuarine systems of India have not received much attention as from the marine environment. The pioneer work on the ecology and seasonal succession of diatom flora of estuarine waters of India was that of Iyengar and Venkataraman (1951) for the Cooum estuary in Madras. Since then biological investigations were carried out by various authors on the planktonic algae of Chilka Lake (Roy, 1954; Devasundaram and Roy, 1954; Patnaik, 1973) and in the Hoogly estuary (Dutta *et al.,* 1954; Gopalakrishnan, 1971). Krishnamoorthy (1954) studied the nutrients in relation to the plankton production in the inshore and the estuarine waters of Porto Novo and Krishnamoorthy and Santhanam (1974) and Santhanam *et al.* (1975) gave a descriptive account of the species distribution and quantitative ecology of the phytoplankton of the same region.

Cochin backwaters have been studied intensively for plant pigments (Quasim and Reddy, 1967), light penetration (Quasim *et al.,* 1968), tidal amplitude (Quasim and Gopinathan, 1969), organic production (Quasim *et al.,* 1969), nutrient cycle (Sankaranarayanan and Quasim, 1969), salinity tolerance of phytoplankton (Quasim *et*

al., 1972), seasonal abundance of phytoplankton (Gopinathan, 1972) spatial and temporal distribution of the phytoplankton (Gopinathan *et al.*, 1974; Joseph *et al.,* 1975), contribution of nannoplankton (Quasim *et al.*, 1974; Vijayaraghavan *et al.*, 1974) and on primary productivity of entire estuarine system (Nair *et al.*, 1975).

1.1.2 Effect of trace metals and vitamins

Micro algae acquire nutrients from their environment in order to sustain growth and reproduction. The term 'trace element' is rather loosely used in the literature to designate the elements, which occur in small concentrations. According to Perkins (1974) the response of marine life to increasing concentration of trace elements in seawater is oligodynamic *i.e.*, stimulatory at low doses and toxic at higher levels. Most of the trace elements are found in living organisms in very low concentration. While some of these are known to have definite functional roles, others are accumulated without any apparent reason. According to Arnon (1950), an element is considered essential for an organism when the organism can neither grow nor complete its life cycle in its absence or it cannot be replaced by any other element and has a direct influence on the metabolism of the organism. In order to evaluate the effects

of trace metals on the aquatic ecosystem, one must have some measurement of the action of these substances to the important components of this ecosystem. Each of the trace metals either singly or in combination along with the major environmental parameters can affect the biota as a whole and the food chain in particular. The idea of employing algae for studying the effects of several metal ions is important because algae are primary producers of the aquatic ecosystems and also because of their simple life cycle.

The post larval stages of crustaceans and spat juvenile stages of bivalves, the diatoms especially *Chaetoceros*, *Skeletonema*, and *Thalassiosira* form the primary food particularly in hatcheries. Rapid multiplication within short period is characteristic feature of the diatom. Media like TMRL (Tung Kong Marine Research Laboratory), 'f' medium (Guillard and Ryther, 1962), Provosoli M edia (Pantastico, 1977) and Conway (Walne, 1974) are suitable for their stock and mass culture. The diatom can tolerate a wide range of salinity from 15-35ppt and temperature range of 20-36^{0}C and so taken for the experimental study.

In aquatic ecosystem especially in the estuarine and nearshore regions the quantity of the trace metal availability is dependent on several factors such as run off water, man made

sources etc. Apart from the existence of these trace metals in natural conditions, there is a discharge point from man made sources. Natural distribution of trace elements in seawater has been compared and discussed by Fabricant *et al.* (1962) and Schutz and Turekian (1965). The concentration of metals found in seawater, open ocean and rivers has been reviewed by Pytkowicz and Kester (1971), Riley and Chester (1971), Preston *et al.* (1972) and Abdullah *et al.* (1972).

The work within our country centered on only on the magnitude of the trace metals dissolved in particulate forms in Indian waters. It has been reported from West coast of India by Sreekumaran *et al.* (1968), Central West Coast of India (Sankaranarayanan and Reddy, 1973), Vellar estuary (Jegatheesan and Venugopalan, 1973; Venugopalan and Ramdhas 1975), Bay of Bengal (Chalapathy Rao and Satyanarayana Rao 1974), Braganca and Sanzgiry (1980), Rajendran *et al.* (1976), Goa waters by Zingde *et al.* (1976), Cochin back waters (Sankaranarayanan and Rosamma Stephan, 1978; Rajendran and Kurian, 1986) and Laccadive sea (Sanzgiry *et al.*, 1979). Inspite of the importance of the algae in aquatic food chains, relatively little attention has been focused upon them. Several reviews on microalgae by Whitton, (1970), Leland and Luoma (1977), Davis (1978, 1983), Leland and

Fielden (1979), Sorentino (1979) and Rai *et al.* (1981) have been published. But they furnish very little information about the elimination of trace elements and vitamins in algal nutrition.

Research on the environmental factors governing the distribution of aquatic life have proved beyond doubt that growth and occurrence of fish depends on a food chain in which the ultimate link is the phytoplankton, comprising mainly diatoms. It is estimated that 20-25% of all organic carbon fixation in the aquatic system is carried out by diatoms. Hence a systematic study of diatoms with special reference to their seasonal abundance and distribution is an essential pre-requisite for the further understanding or the development of shoal fisheries.

The studies regarding the tolerance of microalgae to the nutrients under lab condition were rare. Eventhough there is large number of literature on the effect of higher concentration of trace metals on micro algal culture, the literature regarding the complete elimination of trace elements from the culture was also scanty. Similarly there were not many studies regarding the optimum nutrient requirement of diatom under laboratory conditions. Copper was considered as an essential element for plant growth (Sommer, 1931). Since then it has been proved to act as an important factor in several biochemical process. Trace amount of copper are

essential for metabolic process of algae (Manahan and Smith, 1973; O'kelly, 1974 and Sorentino, 1979). Higher concentrations are toxic and for many years copper sulphate has been used as an algicide to prevent undesirable algal bloom. The biological importance of copper in the sea has been discussed by Lewis and Cane (1982). There have been occasional reports of copper limitations in the natural waters but the evidence is not conclusive. Subba Rao (1981) had accounted the variability of trace metal distribution and the differential growth response of phytoplankton depends on trace metal concentrations. Wolter *et al.* (1984) had reported the influence of low concentration of copper on phytoplankton of natural waters. Considerable variability in sensitivity to copper was evident among certain species of marine diatoms, dinoflagellates, chlorophycean member's etc. causing adverse effect on their growth, survival and development. These observations were documented by several workers (Thomas *et al.*, 1977, 1980; Saifullah, 1978; Gnassia- Barelli *et al.*, 1978, 1982; and Davis, 1980).

Zinc is an important micronutrient for growth and metabolism of the various algae (O'Kelly, 1968) and much work has been done on its metabolism especially in *Euglena* (O'Kelly, 1974). In autotrophic cultures, a linear relation ship between

specific growth rate and internal zinc concentration of cells has been established by Price and Quigley (1966). The earliest estimation of zinc requirement in algae was in *Stichococcus bacillaris* (Eilers, 1926). Thereafter it is assumed universally required by algae. The growth response of the diatom *Nitzschia closterium* (Rosko and Rachlin, 1975), *Chlorella saccharophila* and *Navicula incertia* to selected concentration of zinc which reduced the population growth by 50% after 96 hours of exposure was estimated by Rachilin *et al.* (1982, 1983). The complexation of zinc by metabolites excreted from the marine diatoms and the influence of dissolved organic compounds on toxicity has been highlighted by Fischer and Frood (1980), Fischer and Fabris (1982), Imber and Robinson (1983) and Imber *et al.* (1985). Canterford and Canterford (1980) have expressed the correlation between toxicity and metal speciation in *Ditylum brightwelli*. Gopinathan (1981) correlated the elimination of vitamins from the culture of *Chatoceros* with its growth rate.

The survey of the above literature indicates that more research and viewpoints are necessary for a better understanding of the impact of the elimination of the trace elements and vitamins from Walne's medium and its optimum concentration for the proper growth of microalgae.

The major objective of the study was primarily to determine the effect of the elimination or exclusion of the trace elements and vitamins from the diatom culture especially in *Chaetoceros calcitrans* The elimination of trace metals from the culture and the addition of different concentration of Walne's medium was done in order to determine the role of trace elements in the growth of microalgae and exact day on which maximum utilization of medium was taking place, instead of giving the entire medium on the first day itself. In usual hatchery practices the addition of the culture medium is on the day of inoculation itself. Elimination of trace metal was done to study the role of micro nutrients on the growth of the culture.

Also an attempt has been made to study the possible correlation of the abundance of diatoms in the south west coast of India with the landing of the pelagic fishery resources, especially sardine, mackerel and anchovies, since these fishes are purely diatom feeders. Cooper (1933) has calculated the intensity of phytoplankton production based on the consumption of CO_2 and nutrients and production of oxygen. Later Subrahmanyan (1959) calculated the production of phytoplankton on the west coast of India based on the pigment analysis by Harvey units and indicated that the total landings of the commercial fish represents only a very

small fraction of the total production of phytoplankton. Gopinathan (1981) has accounted 0.2% of conversion efficiency from primary to tertiary production and reported that about 283 million tonnes of carbon is produced annually from the EEZ of India.

Chapter 2

2. Distribution and ecology of diatoms along the south west coast of India in relation to the hydrological parameters

2.1 Introduction

Diatoms are the microscopic plant life of aquatic environment, which constitute the major part of the primary producers synthesizing basic food. Although some work has been done on the taxonomy of the plankton diatoms of Indian seas (Venkataraman, 1939; Menon 1945; Subrahmannyan, 1946; Nair, 1959; Gopinathan 1975 and 1981) very little information is available on the estuarine and marine diatoms and seasonal variations along the south west coast of India. Studies on the ecology of the diatoms in the estuarine and marine waters along the south west coast of India are rare. The pioneer work on the ecology and seasonal variations of diatom flora of India was that of Iyengar and Venkataraman (1951) for the Cooum esturary in Madras. Since then biological investigations have been conducted by various authors on the diatom flora of Chilka Lake (Roy, 1954; Devasundaram and Roy, 1954) and in the Hoogly estuary (Dutta *et al.*, 1954; Roy, 1955; Shetty *et al.*, 1961). It is an established fact

that a combination of different parameters is responsible for the nature of flora and fauna in an ecosystem. Therefore each ecosystem should be taken as a separate entity for the investigation. In Indian coast also there are a number of varying factors with different hydrographic properties both physical and chemical and varying tidal fluctuations. Very little work has been done on the diatoms of the south west coast of India in relation with the hydrological properties. Hence the present study embodies a brief account on the findings of diatoms of the southwest coast of India in relation to the hydrological parameters.

In recent years, the estuarine system of south west coast of India has been a subject of study for various hydrobiological and productivity parameters due to its unique dynamic environmental condition as well as resource potential. Studies on organic production of the estuarine areas showed that the estuarine system is one of the most productive in the tropical environment. According to various authors on their studies in different estuarine systems on variation and distribution of phytoplankton and factors effecting its production has revealed that the standing crop in terms of chlorophyll, biomass, total cell count and primary production vary from place to place and time to time as a results of inflow of fresh water from the rivers and seawater from the inshore areas.

In their earlier investigations, Gopinathan *et al.* (1974) studied the usefulness of chlorophyll *a* in relation to the phytoplankton counts as a measure of phytoplankton abundance by using the correlation coefficient and found from the analysis of co-variance that a common relationship exist between phytoplankton and chlorophyll *a* in Cochin estuary.

Productivity indices such as chlorophyll, biomass and total cell count cannot independently give a true picture of standing crop due to inherent drawbacks in each method. Changes in the environment and place of sampling also affect the hydrological and productivity parameters. Hence an attempt has been made to determine the relationship of various hydrological parameters affecting diatom production.

Area of Study

Along the southwest coast of India three stations were selected namely Thalassery, Cochin and Vizhinjam. Samplings were done monthly during the period 2001-2002 and collected the data related to the hydrobiological and productivity parameters of diatoms along these regions. Fig.1 represents the map of the study area.

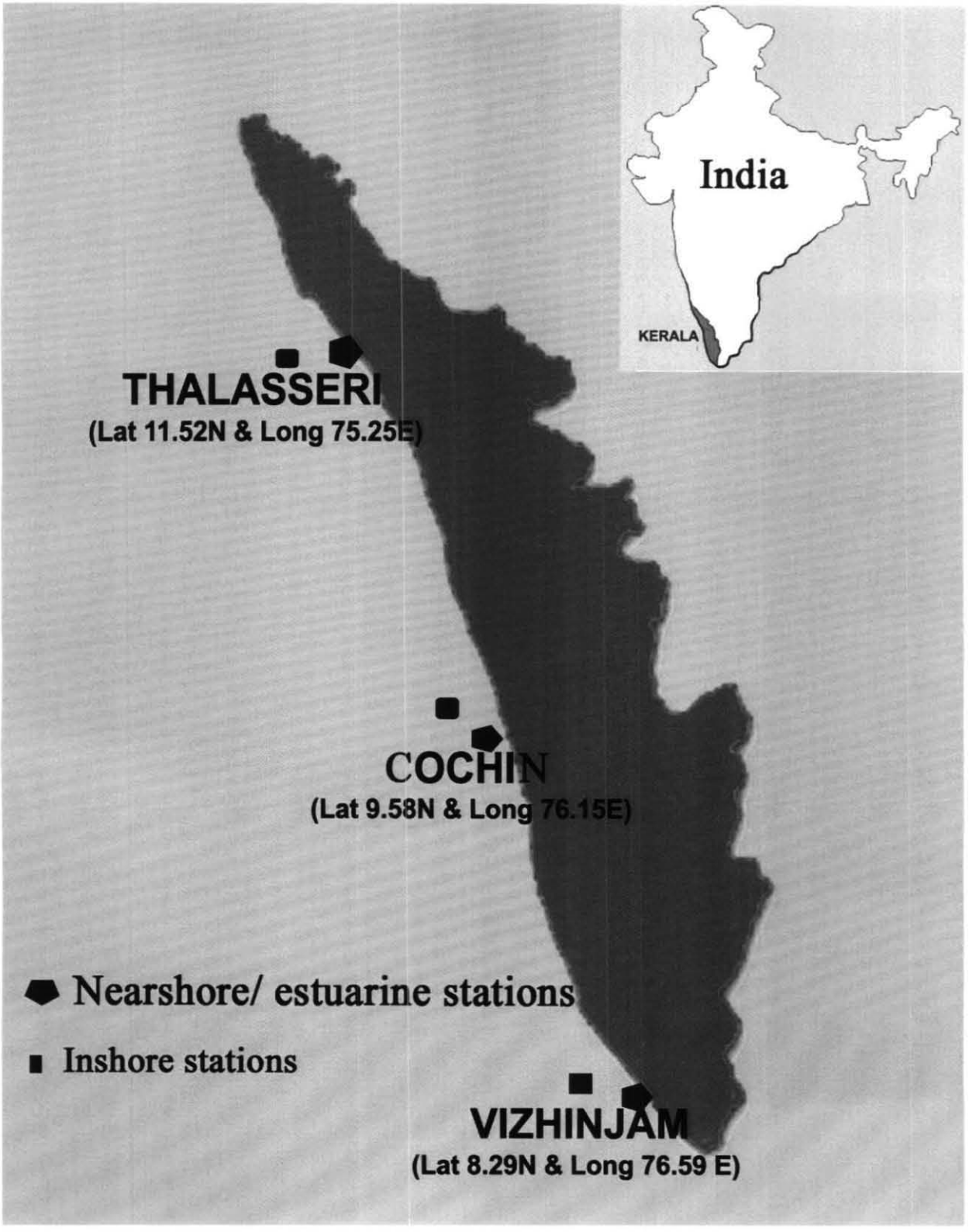


Fig. 1. Map of study area

2.2 Material and methods

2.2.1 Diatoms in the estuarine/nearshore and inshore environments

For quantitative and qualitative investigations, one litre of water was collected from the surface of the nearshore (2-3m) and inshore (10-15 m) areas of the three fixed stations. After settling, one ml of sample was counted along with the identification of the organism up to species level. Duplicate samples were filtered using Millipore filters and the filter paper was treated with 90% acetone and the pigments, chlorophylls were measured using the spectrophotometer according to Strickland and Parsons (1972).

Water samples were also analyzed for the physico-chemical parameters. Determination of salinity, dissolved oxygen and nutrients such as nitrite, nitrate, phosphate and silicate were done following the methods of Strickland and Parsons (1972). Light and dark bottle oxygen technique (Garder and Gran, 1927) was employed for the estimation of primary production. Samples collected were preserved in 4% formalin for the quantitative and qualitative estimation of diatoms.

2.2.2 Diversity indices

Diversity indices were also studied using the Shannon and Weaver (1949) formula with the data collected from the three fixed stations.

2.2.3 Statistical analysis

Statistical analysis especially regression analysis was conducted using SPSS 10.00 version for windows to study the various hydrological parameters contributing the diatom population of the three stations.

2.3 Results

Qualitative studies

During all the seasons the phytoplankton was mainly composed of diatoms. Along Thalassery region *Biddulphia* and *Coscinodiscus* were present almost throughout the year. During the pre monsoon period species of *Fragilaria, Melosira, Nitzschia, Pleurosigma* were dominant and abundance of *Coscinodiscus, Melosira, Nizschia, Pleurosigma, Thalassiosira* etc. were found during monsoon period while *Biddulphia, Coscinodiscus, Skeletonema* and *Thalassiosira* were dominant during post monsoon period. *Cheatoceros* was dominant during the month of January, *Coscinodiscus* during February- April, August, - December, *Nitzschia* during May, *Leptocylindrus* during June,

Melosira during July and *Skeletonema* during November. Altogether 54 species were identified at Thalassery.

According to Quazim *et al.* (1969), Gopinathan *et al.* (1974) and Joseph *et al.* (1975) on their independent studies on the phytoplankton in Cochin backwaters, the standing crop vary from year to year due to meteorological and other environmental factors. In the present study during the pre monsoon season, diatoms dominated along Cochin estuarine area were species of *Coscinodiscus*, *Pleurosigma*, *Melosira*, *Skeletonema*, *Thalassiothrix* and *Fragilaria*. While in the monsoon period, species of *Thalassiosira*, *Thalassionema*, *Leptocylindrus* and *Asterionella* were abundant. During the Post monsoon period species of *Rhizosolenia, Thalassiothrix, Thalassiosira, Skeltonema* and *Chaetoceros* were noted along the Cochin area. In this particular period, *Thalassiothrix* was dominant during January, *Coscinodiscus* during February to May and October, *Leptocylindrus* during June, *Nitzschia* during July and September, *Pleuroshigma* during August, *Skeletonema* during November and *Thalassiosira* during December. Species of *Coscinodisus, Melosira, Pleurosigma* and *Skeletonema* occurred throughout the year. About 65 diatoms were identified along the Cochin estuary.

At Vizhinjam area, the abundance of *Asterionella, Biddulphia, Fragilaria, Pleurosigma, Thalassionema, Melosira,* were found through out the year. During pre monsoon period, diatoms dominated were species of *Melosira, Biddulphia, Asterionella* and *Pleurosigma*, while during monsoon period species of *Asterionella, Coscinodiscus, Fragileria, Pleurosigma, Skeletonema, Thalassiothrix* and *Thalassiosira* were abundant. Abundance of the *Chaetoceros, Asterionella, Hemidisus, Stephanopyxis, Thalassothrix* and *Thalassiosira* were observed in post Monsoon period. During January *Chaetoceros* was dominant, *Coscinodiscus, Thalassionema* during February, *Biddulphia* during March and May, *Asterionella* during April, *Thalassionema* during June, *Skeletonema* during July, *Thalassothrix* and *Thalassiosira* during August, *Thalassionema* during September, *Coscinodiscus, Hemidiscus, Thalassiosira* during the months of October, November and December respectively. Along the nearshore area of Vizhinjam a total of 62 diatoms were identified during the study period.

Along Thalassery inshore area *Thalassiothrix frauenfeldii* was dominant during the month of January, species of *Coscinodiscus* was dominant during the months of February to September, *Thalassionema* during June, October and November,

Pleurosigma during July and August, *Melosira* during December. Abundance of species of *Coscinodiscus, Fragilaria, Melosira, Nitzschia, Pinnularia, Pleurosigma* and *Skeletonema* was observed during the post monsoon period. But during the period of monsoon, species of *Coscinodiscus, Melosira, Nitzschia Pleurosigma, Skeletonema, Thalassionema* and *Thalassiothrix* showed their abundance. Post monsoon period was rich with species of *Biddulphia, Coscinodiscus Navicula, Pleurosigma, Skeletonema, Rhizosolenia, Thalassiothrix* and *Thalassiosira*. Here 50 different diatom species were identified.

Cochin area was rich with species of *Coscinodiscus, Thalassiosira Nitzschia, Pleurosigma, Skeletonema, Asterionella, Melosira and Biddulphia* during pre monsoon season. The monsoon season was having the abundance of *Leptocylindrus, Thalassionema, Thalssiosira, Pleurosigma, Coscinodiscus, Skeletonema, Nitzschia* and *Rhizosolenia. Thalassionema, Thalassiothrix, Coscindiscus, Synedra*, and *Melosira* were common during the post monsoon period. *Species of Asterionella, Biddulphia, Coscinodiscus, Melosira, Navicula, Nitzschia, Pleurosigma,* and *Thalassionema* were observed throughout the year. *Chaetoceros* was dominant during January, *Coscinodiscus* during February, March and December, *Asterionella* showed its

dominance during April, *Thalassionema* during May, June and October, *Pleurosigma* during July- September and *Synedra* during November. Along the inshore areas of Cochin about 43 diatoms were observed during the study period.

Along Vizhinjam area, diatoms which occur through out the year were species of *Biddulphia, Coscinodisucs, Fragilaria, Melosira, Nitzschia, Pleurosigma, Rhizosolenia Skeletonema, Thalassionema* and *Thalassiosira*. During pre monsoon period species of *Coscinodiscus, Thalassiosira, Skeletonema, Nitzschia, Melosira, Asterionella, Thalassionem* and *Biddulphia* were found. During monsoon period abundance of the species of *Thalassionema, Thalassiothrix, Coscinodiscus, Pleurosigma, Asterionella, Nitzschia* and *Navicula* were observed. But during post monsoon period, species of *Synedra, Thalassionema, Coscinodiscus, Pleurosigma, Melosira, Thalassiosira, Asterionella, Cheatoceros, Navicula, Skeletonema* and *Thalssiothrix* were abundant. Species of *Asterionella* showed it's abundance during January and May, *Coscinodiscus* was abundant during February, March and December, *Nitzchia* during April, *Thalassionema* during June, *Pluerosigma* during July, August and September and *Synedra* during December. A total of 54 species of diatoms were noted during the tenure of the study at Vizhinjam inshore area.

Diatom population

Corresponding to primary production and chlorophyll *a* the total cell count also showed evidences of seasonal and spatial variations. The total number of diatom cells also indicated peak periods during post monsoon in the regions of Thalassery and Cochin nearshore/estuarine areas, 11460-46450 and 11050-75810 cells/l respectively. But in Vizhinjam area the diatom population was high during the monsoon season, 20840-71950 cells/l.

Total cell counts showed secondary peak during the monsoon period along Thalassery, 24890-33350 cells/l, while that at Cochin was during pre monsoon period. The secondary peak of diatom population observed at Vizhinjam was during post monsoon season.

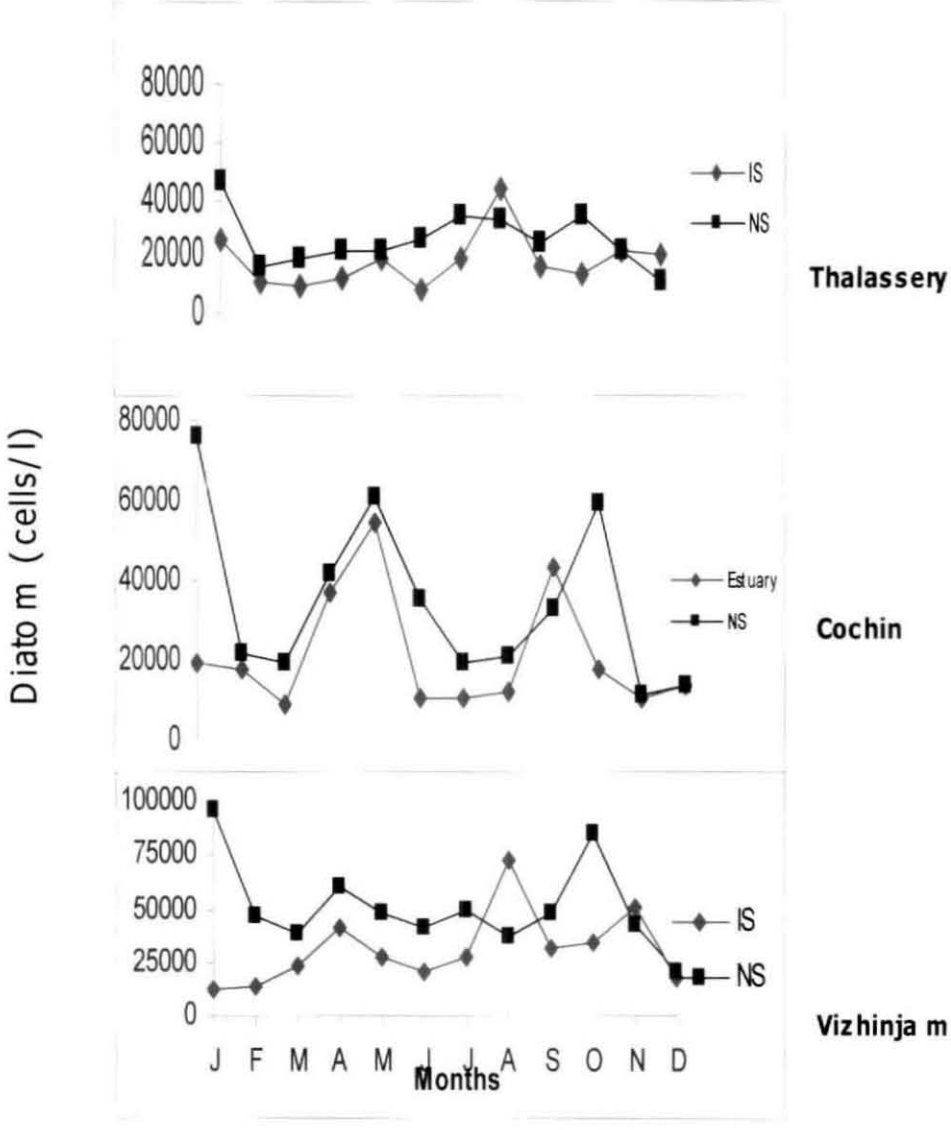
Low cell concentration was observed in the months of February –March and October – January along the Cochin estuary. Diatoms are seen more on the monsoon period and secondary peak of the same during post monsoon period in all the three stations.

Along the inshore area of Thalassery, monsoon season was with maximum concentration of diatoms, 8150-43400 cells/l, followed by pre monsoon season with a concentration ranging between 13600-26300 cells/l, and least was during the post monsoon season, 9520-18520 cells/l.

Cochin station showed the maximum concentration of diatoms during the monsoon season followed by the pre monsoon and post monsoon respectively. The diatom concentration during the monsoon was 10070-54190 cells/l while that for the pre and post monsoon was in the range of 9090-37000, 11050-18970 cells/l respectively.

At Vizhinjam, highest cell concentration was noticed during the post monsoon season with a range of 19970-96400 cells/l, followed by monsoon season with 36780-49320 cells/l and least during the pre monsoon season 38590-46330 cells/l. (Fig.2.1)

Fig.2.1 *Diatom population of different stations*



(Pooled values of 2001-2002)

Total percentage of diatoms

From the total number of phytoplankton, the diatom percentage was calculated. It was found that along the nearshore area of Thalassery the total percentage of diatom was 90.3 for pre monsoon season, while that for Cochin and Vizhinjam were 90, 91% respectively. The highest percentage was observed during the

monsoon period along the three stations, 91, 91 and 90% respectively for Thalassery, Cochin and Vizhinjam. Lower percentage was observed during the post monsoon season. (Table 2.1)

Table 2.1 Percentage of diatoms along Nearshore/Estuarine area

Station	Nearshore /Estuarine area		
	Premonsoon	Monsoon	Post monsoon
Thalassery	90.3	91	89
Cochin	90	91	87
Vizhinjam	91	90	89

The highest percentage of diatoms in the inshore area was noticed along the Thalassery and Cochin during post monsoon and monsoon period, 89% and 92% respectively. While that for Vizhinjam was during Monsoon period (90%). Secondary peak was observed during Monsoon period along three stations. It was noticed that throughout the entire season, the diatoms contributed more than 85% of the total phytoplankton population. (Table 2.2)

Table 2.2 Percentage of diatoms along inshore area

Station	Inshore area		
	Premonsoon	Monsoon	Post monsoon
Thalassery	86	89	89
Cochin	89	90	92
Vizhinjam	81	89	87

Diversity indices

Species diversity is a basic measure of community structure and organization and most important parameter to understand the health status of the ecosystem. The diversity index gives a measure of how the individuals in a community are distributed. Information on the species diversity, richness, evenness and dominance evaluation on the biological components of the ecosystems are essential to understand detrimental changes in environs or deterioration of water quality

In the context of the global loss of thousands of species as a result of pollution and habitat destruction, assessments of species diversity and richness are highly needed. Such studies assist the environmental biologists to predict where and how many species go extinct so that certain effective measures may be taken to conserve them. Here an attempt has been made to study the species diversity following the method of Shannon and Weaver (1949).

Diversity indices of Thalassery showed that monsoon season is having the maximum abundance 1.36 and dominance (0.94) of diatom, followed by the post monsoon period [1.28] and pre monsoon [0.84] respectively [Table 2.3].

Table 2.3 Diversity indices along the nearshore area of Thalassery

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	1492	2613	2183
Richness index	1.2	1.36	1.28
Evenness index	0.835	0.94	0.84
Shannon's index	1.95	1.99	1.977

While along the Cochin estuarine area showed maximum richness and abundance during the monsoon itself [1.82] and [0.825] respectively followed by the post monsoon season [1.76, 0.715] (Table 2.4).

Table 2.4 Diversity indices along the estuarine area of Cochin

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	2456	6566	5994
Richness index	1.24	1.82	1.76
Evenness index	0.63	0.825	0.715
Shannon's index	1.65	1.895	1.75

The diversity indices along the Vizhinjam station showed highest values for abundance and richness during the post monsoon season, 2.46 and 0.88 respectively followed by monsoon season with values of 2.39 and 0.80 (Table 2.5).

Table 2.5 Diversity indices of nearshore area of Vizhinjam

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	3192	4505	5326
Richness index	1.775	2.39	2.46
Evenness index	0.865	0.80	0.88
Shannon's index	2.345	2.435	2.45

Diversity indices were studied for the diatoms of inshore areas. At Thalassery the abundance and richness was higher during the monsoon season with a value of 2.02 for the richness index and 0.8 for the evenness index, this was followed by the post monsoon season (Table 2.6).

Table 2.6 Diversity indices along inshore area of Thalassery

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	2019	3075	2841
Richness index	1.64	2.02	1.87
Evenness index	0.81	0.8	0.8
Shannon's index	2.11	2.22	2.18

At Cochin, the abundance and richness was noticed during the post monsoon period 2.15 and 0.84 respectively, followed by the premonsoon period with a value of 2.1 for richness index and 0.84 for evenness index (Table 2.7).

Table 2.7 Diversity indices along inshore area of Cochin

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	6158	5252	6514
Richness index	2.1	1.98	2.15
Evenness index	0.84	0.81	0.84
Shannon's index	2.34	2.31	2.46

At the same time the maximum abundance and richness was noticed during the post monsoon period followed by the pre monsoon at Vizhinjam with the values of richness index and evenness index 2.52 and 2.3, 0.85, 0.83 for the post monsoon and pre monsoon season respectively. [Table 2.8]

Table 2.8 Diversity indices along inshore area of Vizhinjam

	Premonsoon	Monsoon	Post monsoon
No. of Individuals	6260	5409	6975
Richness index	2.3	1.81	2.52
Evenness index	0.83	0.78	0.85
Shannon's index	2.39	2.34	2.5

Productivity

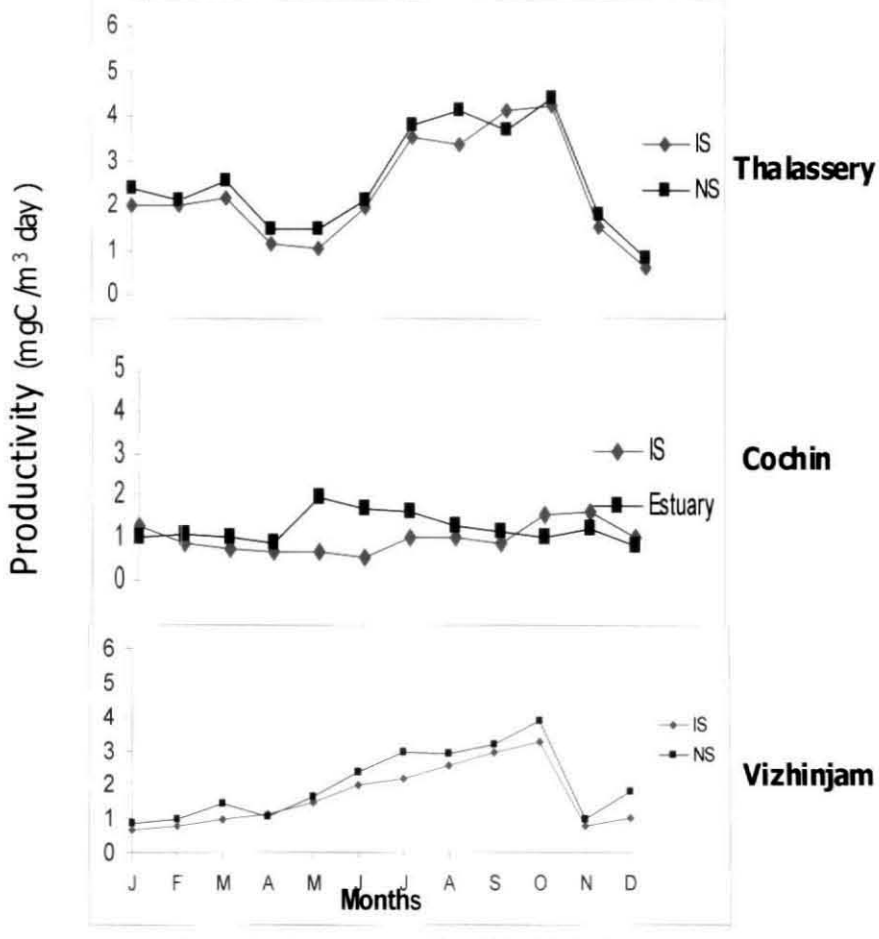
In the present investigation, two seasonal peaks of primary production were noted along the estuarine area of all the three stations. The range of production for Thalassery, in the pre-monsoon period was 01.45- 2.56 mgC/l/day and for monsoon period, it was 2.13-4.1 mgC/l/day while that for post monsoon period was 0.79-4.36 mgC/l/day indicating post monsoon months are most productive season.

The productivity range in the station Cochin was 0.85-1.94 for pre monsoon and 1.13-1.71 mgC/l/day for monsoon and 0.0.79-1.25 mgC/l/day for post monsoon season. Here also there was high productivity observed in the pre monsoon season. Productivity in the Vizhinjam area showed slight fluctuations. The productivity during the pre monsoon was 0.63-1.45 mgC/l/day and 2.36-3.17 mgC/l/day for monsoon while that for post monsoon was 0.98-3.86 mgC/l/day.

Along the inshore area gross primary productivity showed a lower value in comparison with the estuarine region in all the three stations. The Thalassery region showed a peak value during the post monsoon period and it was 0.59-4.22 mgC/l/day while that for the monsoon and post monsoon was 1.97-4.1 mgC/l/day and 1.02-2.15 mgC/l/day respectively. The peak was observed in the post

monsoon season at Cochin inshore area. Here the value observed during monsoon was in the range of 0.57-0.99 mgC/l/day and that for the pre and post monsoon was 0.07-0.87 mgC/l/day and 1.02-1.65 mgC/l/day respectively. Along the Vizhinjam area values during the post monsoon period was high and it was 0.65-3.26 mgC/l/day while that for the pre monsoon and monsoon was 0.76-1.44 mgC/l/day and 1.97-2.56 mgC/l/day respectively. Fig. 2.2 represents the details of productivity in the nearshore/estuarine and inshore areas of three stations.

Fig.2.2 ***Productivity of different stations***



(Pooled values of 2001-2002)

Chlorophylls

In the present study Chlorophyll *a, b,* and *c* were measured at all the three stations. Since Chlorophyll *a* is one of the major indices of the standing crop of phytoplankton, the estimation of this along with productivity will give a general idea of the variation in the magnitude of production.

Along the Thalassery area the chlorophyll *a* was higher during the monsoon season 1.04-1.87 mg/m^3 and that for the pre and post monsoon were 0.43-1.35 mg/m^3 and 1.04-1.25 mg/m^3 respectively. The chlorophyll *b* was higher during the monsoon *i.e.*, 0.99-1.13 mg/m^3 and that for pre monsoon and post monsoon were 0.12-0.65 mg/m^3 and 0.03-0.65 mg/m^3 respectively. Chlorophyll *c* was higher during the monsoon period with a value of 0.78-1.63 mg/m^3. Table 2.9 represents the chlorophyll values along the Thalassery area.

Table 2.9 Mean Chlorophyll values [mg/m^3] of Thalassery nearshore area. (Pooled values of 2001-2002)

	a	*b*	*c*
January	1.25±0.35	0.65±0.02	0.32±0.03
February	0.43±0.34	0	0
March	0.81±0.25	0.12±0.05	0.08±0.02
April	1.25±0.35	0.65±0.69	0.32±0.42
May	1.35±0.46	0.33±0.16	0.79±0.19
June	1.87±0.36	1.06±1.02	0.63±0.89
July	1.5±0.71	1.00±0.02	0.41±0.16
August	1.2±0.07	1.13±0.79	0.97±0.03
September	1.04±0.07	0.99±1.25	0.78±0.31
October	1.04±0.07	0.09±0	0.80±0.23
November	1.07±0.04	0.03±0.03	0.07±0.09
December	0.000	0.00	0.000

At Cochin, chlorophyll *a* value was higher in the post monsoon season, 2.26-9.7mg/m^3. It was 0.99-1.59 mg/m^3 during pre monsoon period and 0.02-2.97mg/m^3 in monsoon period. Chlorophyll *a* values showed a peak during post monsoon period indicating a direct relationship with productivity.

Chlorophyll *b* for pre monsoon period was 0.32-1.58 mg/ m^3, 0.73-0.90 mg/m^3 for monsoon period and 0.2-1mg/m^3 for post monsoon period respectively.

Chlorophyll *c* value was 0.37-1.8 mg/m^3 during the pre monsoon and 0.19-1.32 mg/m^3 during the monsoon and 1.12-3.58 mg/m^3 during the post monsoon season. Here also maximum value

noted during the post monsoon period showing a direct relationship with the productivity. Table 2.10 shows the chlorophyll values of Cochin estuarine area.

Table 2.10 Mean Chlorophyll values [mg/ m^3] of Cochin estuarine area (Pooled values of 2001-2002)

	a	b	C
January	2.26±1.27	0.20±0.26	2.32±0.49
February	1.00±0.34	0.56±0.40	0.40±0.20
March	1.26±0.48	0.37±0.16	0.37±0.16
April	0.99±0.03	1.58±0.53	1.58±0.53
May	1.59±0.82	0.32±0.14	0.32±0.74
June	0.02±0.03	0.1±0.12	0.19±0.1
July	1.26±0.06	0.73±0.26	0.67±0.47
August	2.97±2.62	0.85±0.16	1.32±0.74
September	1.20±0.44	0.90±0.90	0.29±0.40
October	9.7±0.26	0.66±0.46	3.58±0.60
November	1.97±0.91	1.00±0.15	3.15±0.21
December	6.24±0.35	0.23±0.02	1.12±1.02

At the same time the chlorophyll *a* value for the Vizhinjam area was higher during the monsoon season 3-10.86 mg/ m^3 and that for the pre and post monsoon period were 0.16-2.32 mg/m^3, and 0.04-0.35 mg/m^3 respectively. Chlorophyll *b* values showed a peak during the pre monsoon season *i.e.*, 0.04-2.54 mg/ m^3 while for the monsoon and post monsoon period were 0.01-0.19 mg/m^3 and 0.01-0.46 mg/m^3 respectively. Chlorophyll c values also showed the peak during the pre monsoon *i.e.*, 0.01-6.58 mg/m^3 and that for the monsoon and post monsoon were 0.30-1.10 and 0.01-

1.35 mg/m^3 respectively. Table 2.11 represents the chlorophyll values along the Vizhinjam nearshore area.

Table 2.11 Mean Chlorophyll values [mg/m^3] of Vizhinjam nearshore area (Pooled values of 2001-2002)

	a	*b*	*c*
January	0.04±0.02	0.11±00	0.05±0.06
February	0.21±0.05	0.05±0.05	0.1±0.01
March	0.16±0.03	0.04±0.04	0.13±0.17
April	2.32±0.51	2.54±0.82	6.58±2.33
May	1.00±0.16	0.20±00	0.30±0.16
June	3.00±1.46	0.19±0.06	0.33±0.08
July	6.60±0.90	0.14±0.06	1.10±1.42
August	3.21±0.82	0.01±0.13	0.30±0.06
September	10.86±1.24	0.09±0.11	0.01±1.30
October	0.35±0.09	0.01±0.07	0.01±0.01
November	0.14±0.06	0.10±0.14	0.08±0.10
December	0.05±0.04	0.46±0.13	1.35±0.49

At Thalassery inshore area, chlorophyll *a* was in the range of 2.66-5.76 mg/m^{3}during the pre monsoon, 0.32-10.29 mg/m^3 during the monsoon and 2.80-10.38 mg/m^3 during the post monsoon season. Chlorophyll *b* was in the range of 0.62-2.58 mg/m^3 during pre monsoon, 0.93-0.97mg/m^3, 0.2-1.69 mg/m^3 during monsoon and post monsoon respectively. While chlorophyll *c* was in the range of 0.47-2.45 mg/m^3, 0.22-1.76 mg/m^3 and 1.43-3.88 mg/m^3 during the premonsoon, monsoon and post monsoon seasons (Table 2.12).

Table-2.12 Mean chlorophyll values [mg/m^3] of Thalassery inshore area (Pooled values of 2001-2002)

	a	b	c
January	3.68±4.03	0.2±0.20	2.49±1.77
February	5.76±5.46	0.67±0.10	0.47±0.14
March	4.89±4.88	0.86±0.08	2.45±0.37
April	2.66±0.95	2.58±0.78	2.40±0.14
May	5.80±6.22	0.62±0.11	1.87±0.36
June	0.32±0.31	0.2±0.11	0.22±0.02
July	6.08±5.96	0.93±0.06	0.77±0.19
August	10.29±9.66	0.97±0.09	1.76±0.22
September	5.74±2.41	0.94±0.05	0.31±0.24
October	10.38±9.89	0.88±0.12	3.88±1.25
November	2.80±2.60	1.69±0.50	3.17±0.24
December	6.24±6.60	0.29±0.11	1.43±0.23

Chlorophyll *a* also showed peak values during the post monsoon period in all the three stations. Along the Cochin inshore area the chlorophyll *a* was observed in the range of 0.32-1.14 mg/m^3, 1.00-1.57 mg/m^3 and 0.1-1.2 mg/m^3 during the pre monsoon, monsoon and post monsoon respectively. Chlorophyll *b* was in the range of 0.1-0.46 mg/m^3, 0.85-1.11 mg/m^3 and 0.01-0.54 mg/m^3 respectively during the pre monsoon, monsoon and post monsoon season and the chlorophyll *c* in the range of 0.05-0.70, 0.69-0.76 mg/m^3 and 0.01-0.76 mg/m^3 respectively during the pre monsoon, monsoon and post monsoon season (Table 2.13).

Table-2.13 Mean chlorophyll values [mg/m^3] of Cochin inshore area (Pooled values of 2001-2002)

	a	*b*	*c*
January	1.20±0.51	0.54±0.12	0.14±0.08
February	0.32±0.00	0.12±0.01	0.00±0.00
March	0.80±0.37	0.10±0.00	0.05±0.02
April	1.03±0.25	0.46±0.12	0.28±0.14
May	1.14±0.20	0.31±0.10	0.70±0.22
June	1.52±0.58	1.00±0.00	1.59±0.48
July	1.36±0.29	1.00±0.25	1.40±0.06
August	1.57±0.43	1.11±0.13	0.90±0.05
September	1.00±0.01	0.85±0.07	0.69±0.05
October	1.00±.01	0.07±0.02	0.76±0.08
November	1.02±0.53	0.01±0.00	0.07±0.06
December	0.01±0.00	0.14±0.01	0.01±0.00

Along the Vizhinjam inshore area the chlorophyll a was higher during the monsoon period with a range of 3.13-12.42 mg/m^3 while that in pre and post monsoon were 0.03-2.90 mg/m^3 and 0.05-0.37 mg/m^3 respectively. The chlorophyll *b* value was higher during premonsoon with a range of 0.06-2.73 mg/m^3 and that for the monsoon and post monsoon were 0.17-0.27 mg/m^3, 0.11-0.47 mg/m^3 respectively. The chlorophyll *c* value was higher during the pre monsoon with a range of 0.13-7.58 mg/m^{3}and that for the monsoon and post monsoon period were 0.33-1.28 mg/m^3, 0.08-0.91mg/m^3 respectively (Table 2.14).

Table-2.14 Mean chlorophyll values [mg/m^3] of Vizhinjam inshore area (Pooled values of 2001-2002)

	a	*b*	*c*
January	0.04±0.03	0.11±0.00	0.000
February	0.03±0.01	0.00	0.000
March	0.18±0.06	0.06±0.02	0.13±0.02
April	2.90±1.31	2.73±1.03	7.58±0.87
May	1.46±0.70	0.24±0.06	0.38±0.12
June	3.13±0.76	0.27±0.08	0.35±0.12
July	7.09±0.17	0.17±0.61	1.28±0.40
August	3.71±1.16	0.00	0.33±0.07
September	12.42±0.85	0.00	1.24±0.32
October	0.37±0.11	0.11±0.01	0.00
November	0.15±0.05	0.12±0.03	0.08±0.04
December	0.05±0.01	0.01±0.40	0

Environmental factors affecting the diatom production

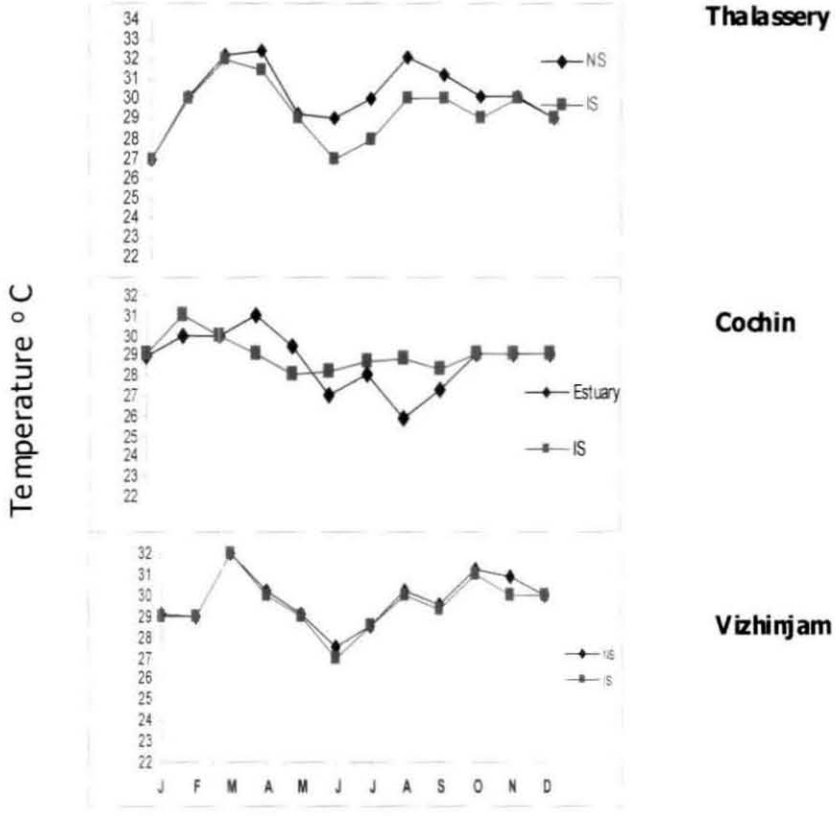
Both physical and chemical factors may influence the production and distribution of the diatoms in an ecosystem. In the present study ,the hydrological factors like pH, salinity, temperature, dissolved oxygen and nutrients such as nitrite, nitrate, phosphate and silicate were estimated in order to make an assessment of their role in the quantitative and seasonal variations of the diatoms.

Temperature

At Thalassery temperature ranged for the pre monsoon period was 29-32.5 ^{0}C, in monsoon it was 29-32 ^{0}C and for post monsoon it was 27-30 ^{0}C. Temperature was maximum during the

pre monsoon months extending upto May and with the onset of monsoon the temperature decreases. During the monsoon and the post monsoon there was low temperature values. The fluctuation in temperature during the pre monsoon was 29.5-31^{0}C and that in the monsoon was 25.9-28^{0}C, while that for the post monsoon was 29 ^{0}C along the Cochin area. In the Vizhinjam area it was 29-32 ^{0}C, for the pre monsoon period, 27.52-30.25^{0}C for the monsoon and 29-31 ^{0}C for the post monsoon. Temperature showed a higher value along the inshore region, it was 28-31^{0}C during the pre monsoon season and 28-29^{0}C during monsoon while it was 29 ^{0}C during the post monsoon along the Cochin region. In the present, study direct relation observed between the productivity and the temperature. But at the Vizhinjam and Thalassery area the high temperature range was recorded during pre monsoon season, with a range of 29-32^{0}C, respectively. The temperature fluctuation was represented in Fig. 2.3.

Fig.2.3 Variation of temperature of different stations



(Pooled values of 2001-2002)

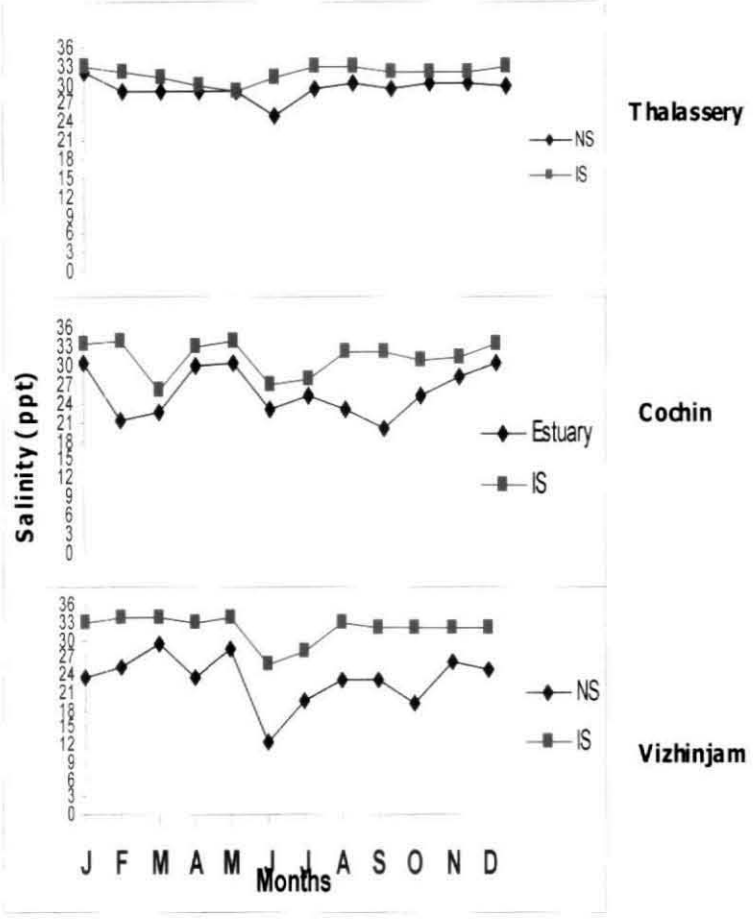
Salinity

The salinity is one of the most important hydrological parameters in an estuary because it regulates the entire biological activities of an ecosystem. Salinity for Thalassery nearshore area was 29 ppt for pre monsoon period, 25-30 ppt for the monsoon and 30-32 ppt for post monsoon. Along the Cochin estuary the salinity range was 21-30 ppt for the pre monsoon period, 20-25 ppt for the monsoon and 25-30 for the post monsoon. At the same time, in the Vizhinjam nearshore area the salinity range was 25-29

ppt for pre monsoon, 12-23 ppt for monsoon and 19-25 ppt for post monsoon. There was a wide range of salinity variation in all the three stations. During the pre monsoon season the salinity was high. But during the monsoon season, the salinity becomes comparatively low because of the entry of the large quantity of the fresh water from the nearby rivers and from the rain fall. Subsequent increase in the salinity was observed during the post monsoon season. The extent of the intrusion of the saline water depends upon the strength of the tidal influx and the fresh water mixing.

Salinity was lower in the monsoon season as in the case of the nearshore area along the three stations. This is due to the dilution of water from the nearby rivers and the rainwater. At Thalassery the highest salinity was noted during the post monsoon period with a range of 32-33 ppt, followed by the monsoon with a range of 31-33 ppt. At the same time the salinity range at Cochin during the pre monsoon period was 26-34 ppt, and that for the post monsoon 30-33.4 ppt. At Vizhinjam also the lowest salinity was during the monsoon period ranging between 26-33 ppt. (Fig. 2.4).

Fig.2.4 *Salinity variation for different stations*



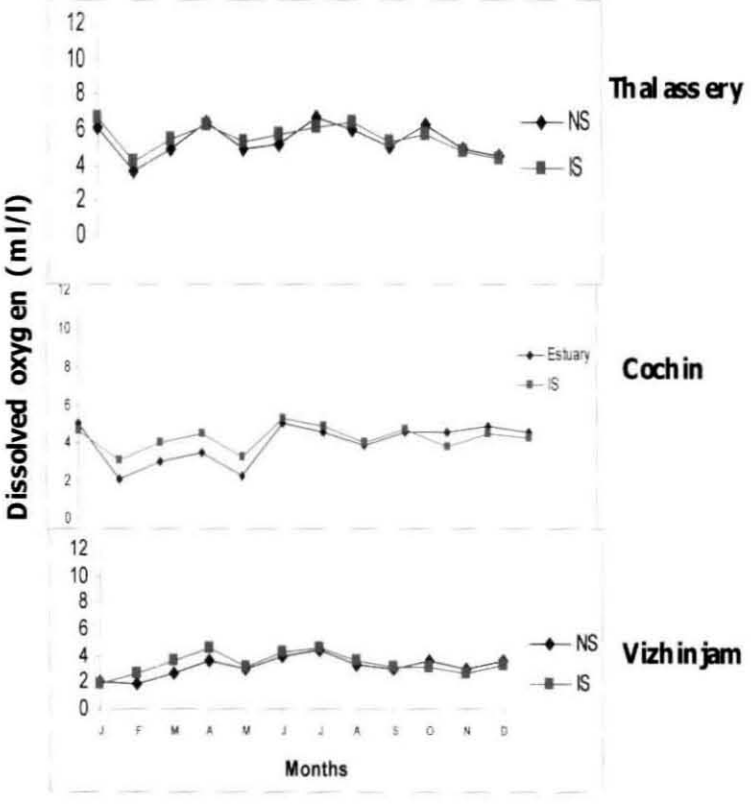
(Pooled values of 2001-02)

Dissolved Oxygen

Along the nearshore area of Thalassery, the dissolved oxygen content was higher during the monsoon period, followed by post monsoon and premonsoon with the values in the range of 5.03-6.65 ml/l, 4.56-6.21 ml/l and 3.69-6.36 ml/l respectively. The same trend was observed along the nearshore areas of Cochin and Vizhinjam. At Cochin the values were 3.89-5.02 ml/l, 4.57-5.01 ml/l and 2.12-3.49 respectively for monsoon, post and pre monsoon seasons.

Along the inshore area of Thalassery the post monsoon season was with high dissolved oxygen, followed by monsoon and premonsoon, values being 4.42-6.67 ml/l, 5.34-6.36 and 4.27-6.25 ml/l respectively. While at Cochin the same trend was observed with values of 3.24-5.27 ml/l, 3.75-4.89 and 3.78-4.64 ml/l respectively. At Vizhinjam inshore area highest value was noted during the monsoon followed by pre and post monsoon. (Fig.2.5).

Fig. 2.5 ***Variation in dissolved oxygen for different stations***



(Pooled values of 2001-02)

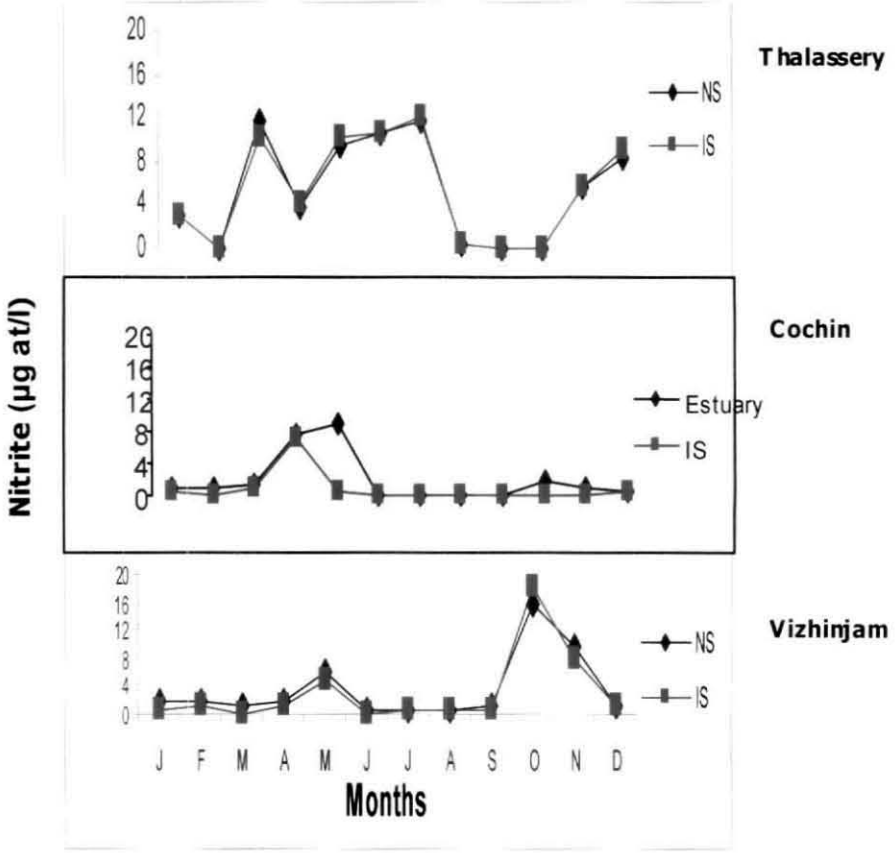
Nutrients

In the present study nutrients such as nitrite, nitrate, phosphate and silicate were estimated. Nitrite value was high in

the pre monsoon season in the Cochin estuarine area 1.02-8.69 µgat/l while in the Vizhinjam and Thalassery it was in the post monsoon period 1.21-15.69 µgat/l and 0.14-8.23 µgat/l respectively.

The nitrite value was higher at Thalassery inshore area during the 0.04-11.87 µgat/l, followed by the pre monsoon 0-11.75µgat/l and post monsoon 0.14-8.23 µgat/l respectively. While at Cochin the highest nitrite value was recorded during the pre monsoon season, 0.2-6.9µgat/l followed by post monsoon and monsoon season 0.06-0.44 and 0.06-0.08 respectively. There was higher values of nitrite during the post monsoon period, followed by the pre monsoon and monsoon with values 0.71-18.75, 0.0-4.73 and 0.28-0.81µgat/l respectively. Fig.2.6 represents the nitrite variation of the three stations.

Fig.2.6 ***Values of nitrite for different stations***



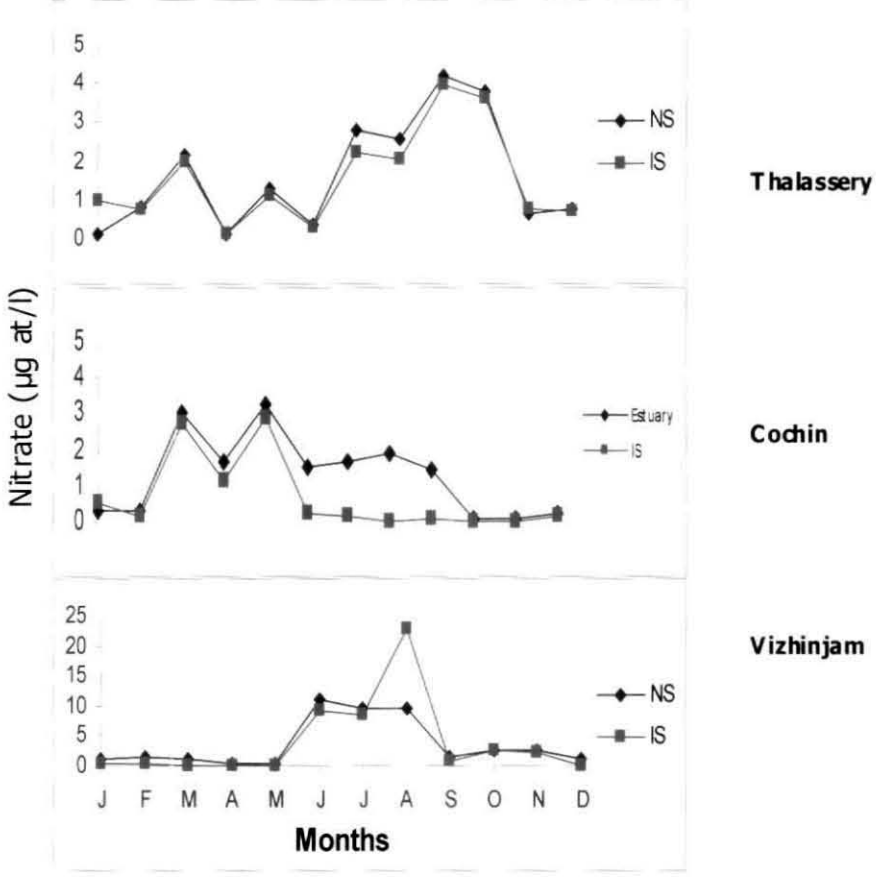
(Pooled values of 2001-02)

The nitrate showed high values during the pre monsoon season at Cochin estuarine area, 0.28-3.26µgat/l and in the Vizhinjam and Thalassery it was in the monsoon period, 1.56-11.2 µgat/l and 0.36-4.16µgat/l respectively.

The nitrate value was highest during the monsoon season at Thalassery and Vizhinjam inshore area, 0.36-4.16 µgat/l, 0.6-22.87 µgat/l respectively, but it was higher during the pre monsoon season at Cochin inshore area 0.15-2.9 µgat/l and during the monsoon and post monsoon period was 0.01-0.19 and 0.15-2.9 µgat/l respectively. The value of nitrite during the pre monsoon

season at Thalassery and Vizhinjam was in the range of 0.12-2.14 and0-0.27 µgat/l respectively, while that for the post monsoon season was in the range of 0.74-3.79 and 6.02-2.45 µgat/l respectively (Fig. 2.7).

Fig 2.7 ***Values of nitrate for different stations***



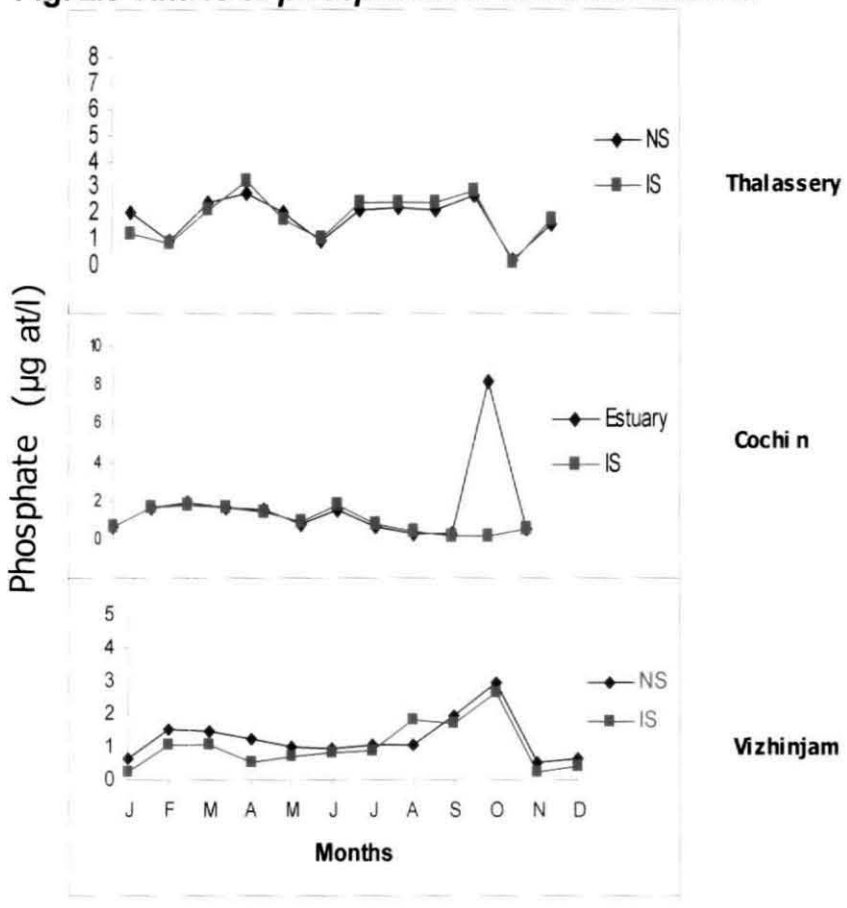
(Pooled values of 2001-02)

Values of phosphate the showed peak during the pre monsoon period at Cochin and Thalassery, 1.54-1.85 µgat/l and 2.1-2.68 µgat/l respectively, while it was in the post monsoon season at Vizhinjam, 0.52-2.96 µgat/l. An abnormal increase was noticed along the nearshore area of Cochin during the month of

October, may be due to the influx of agriculture fertilizers from the Vembanad lake.

The phosphate values also showed fluctuation in its concentration along the inshore areas of three stations. At the inshore area of Thalassery the higher values were noted during the pre monsoon season, 0.82-13.39 µgat/l, followed by the post monsoon and monsoon season, 0.13-2.88 µgat/l and 1-2.37 µgat/l respectively. But at Cochin inshore area, the higher values of phosphate was recorded during the pre monsoon, 1.36-1.67µgat/l, followed by the monsoon and post monsoon with values in the range of 0.32-1.67 µgat/l and 0.15-0.56 µgat/l respectively. The phosphate values along Vizhnjam was 0.54-1.05 µgat/l, 0.82-1.82 µgat/l and 0.22-2.65 µgat/l respectively for the pre monsoon, monsoon and post monsoon seasons (Fig 2.8).

Fig. 2.8 *Values of phosphate for different stations*



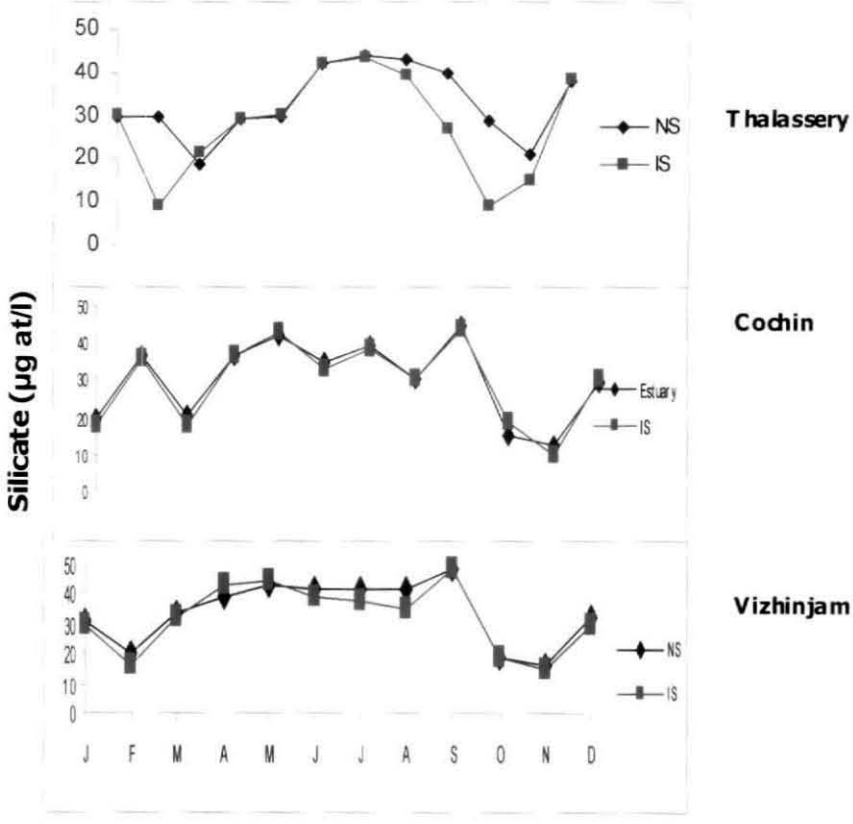
(Pooled values of 2001-02)

In contrary to nitrate nitrite and phosphate, the silicate values showed high during the monsoon season in all the nearshore area of the three stations coinciding with the chlorophyll peak. Along Cochin it was 30.52-45.1 µgat/l, while at Vizhinjam and Thalassery it was 42-48.69, 40.11-44 µgat/l respectively. Fig. 2.9 represents the variation in the silicate contents in all the three nearshore stations.

Silicate values were higher during the monsoon period along the inshore areas of the three stations. At Thalassery it was 9.04-30.38 µgat/l, 27.03-43.77 µgat/l and 9.04-38.73 µgat/l

respectively for the pre monsoon, monsoon and post monsoon season. At the same time the values were 16.43-45, 35.25-49 and 15.08-30.38 µgat/l along Vizhinjam. While at Cochin it was 18.06-42.9, 30.01-44.01 and 10.22-30.22 µgat/l respectively in the three seasons.

Fig.2.9 ***Values of silicate for different stations***



(Pooled values of 2001-02)

Regression analysis

It is well known that combinations of different parameters are responsible for the nature and distribution of diatoms in an aquatic ecosystem. Hence in order to study the influence of various hydrological parameters, a statistical analysis was done

using the data from the nearshore and inshore areas of the three selected stations. A regression analysis was carried out in SPSS, the hydrological parameters such as temperature, salinity, oxygen and nutrients such as nitrite, nitrate, phosphate and silicate were taken as independent variables and primary productivity as the dependent variable. From the regression analysis it can be seen that the contributing variables are different for the different stations of nearshore area showing that they stand independently from each other.

Along the nearshore area of Thalassery the factors affecting the diatom population were, nitrite and salinity and phosphate. An inverse relation was observed for the nitrite and phosphate. At the same time the various hydrological parameters contributing the diatom population along the Cochin nearshore were phosphate, silicate and temperature in the order of their significance. Here the silicate showed an inverse relationship while phosphate and silicate showed a positive relationship.

At Vizhinjam, the important variables in the order of significance are nitrate and temperature only. No significant relation was noticed with other variables and diatom population. The regression equations for the three nearshore areas were represented in Table 2.15.

Table 2.15 Regression equation for the three nearshore areas

Name of the station	Regression equation	SEE	R^2	F	d.f.
Thalassery	D=22457.24-528.42(NO_2)+685.875(S‰)-1325.13(PO_4)	557.9	0.8147	9.53**	4,7
Cochin	D=4314.80+1257. 58(PO_4)-76.201(SiO_3)+1852.048(T)	518.5	0.8139	7.61*	4,6
Vizhinjam	D=2830.47-666.49 (NO_3)+312.75 (T)	246.2	0.8421	9.94**	4,7

* Significant at 5% level, ** significant at 1% level

Along the inshore area of Thalassery the variables contributing the diatom population were phosphate and temperature. Here a positive relationship was there for the two variables. At Cochin the important variables contributing the diatom population were nitrite, nitrate and salinity. Here also positive relationship was noticed. While at Vizhinjam the variable in the order of significance are nitrate, silicate and salinity. Here an inverse relationship was noticed with the silicate (Table 2.16).

Table 2.16 Regression equation for the three inshore areas

Name of the station	Regression equation	SEE	R^2	F	d.f.
Thalassery	D=18992.329+434.560 (PO_4)+745.478(T)	546.20	0.8203	7.53*	5,7
Cochin	D=79879.062-889.540(NO2)+41166.485(NO_3)+)+348.987 (S‰)	561.07	0.7988	7.95*	4,6
Vizhinjam	D=2946.45+271.695(NO_3) - 375.456(SiO_3)+ 1746.415(S‰)	246.2	0.8421	9.14**	4,7

* Significant at 5% level, ** significant at 1% level

2.4 Discussion

Results of the present study revealed that the estuarine diatoms showed two peak periods, one during the monsoon and other during the post monsoon. Gopinathan (1975) pointed out that majority of Indian estuarine diatoms revealed their abundance during May-Agust and secondary peak during October-December. At Trivandram the peak period was from January –May (Menon, 1945), at Calicut it was from May - September (Subrahmanyan, 1959 a) and at Bombay it was from September to February (Gonsalves, 1947). From the observations of various workers it was clear that there is variation from year to year in the distribution of diatoms due to the hydrological and environmental parameters.

Diatoms along the inshore area of Cochin showed the primary peak during the monsoon while that along Vizhinjam and Thalassery showed its abundance during the post monsoon season. Secondary peak along Cochin was during the post monsoon that for Vizhinjam and Thalassery was during the monsoon and pre monsoon respectively. However in all the three stations the diatom concentration was more in the inshore area.

Productivity was higher during the post monsoon period along Cochin estuary but it was higher during the monsoon period in other two stations. Also, there is no direct relation

noticed between the cell count and productivity. According to Selvaraj *et al.* (2003) the gross primary productivity of Cochin backwater is higher during the post monsoon period. Further, Quazim *et al.* (1969) stated that there was high rate of organic production in the backwater area than the inshore. They also pointed out that the fluctuation may not be consistent from year to year due to prevailing environmental conditions.

However, Selvaraj *et al.* (2000, 2003), reported that the productivity is greater along the inshore area than the estuarine area. In the present study, along the three stations, the productivity was higher along the nearshore area than the inshore region. The fluctuation was largely due to relative dominance of one or more species of phytoplankton which some times forms the bloom.

Chlorophyll values did not show any possible relationship corresponding to the variation in the cell count and productivity in all the three estuarine regions. Selvaraj [2003] reported that this may be due to variation in the chlorophyll content of different species constituting the total phytoplankton biomass and turbidity is the another reason for low productivity.

The probable explanation for the high value of chlorophyll *c* may have resulted due to the pigment coming from the stirred up mud from the bottom, which contained more

chlorophyll degradation products. Vallenttyne (1965) and Krey (1958) have indicated the role, which the dead chlorophyll plays in the estimation of the phytoplankton pigments of the coastal waters. From the pigment analysis it was very clear that the estuarine water is characterized by the large quantity of inorganic and organic particulate matter. Since the water masses are constantly renewed by the inflow of the seawater it is not possible to estimate how much pigment came from living organisms alone. The pigment stock has been found to vary considerably from place to place and from time to time due to tidal rhythm.

In temperate countries, temperature is known to play an important role in determining the fluctuation of diatom population and their distribution. In tropical estuaries, the temperature never acts as a limiting factor for the production of diatoms. In the different estuarine systems of India, though there are marked changes in the temperature, their influence by itself has no direct bearing for the multiplication of the new diatom cells. According to Gopinathan (1975) the temperature variation along Cochin back water was very low with a range of 4°C. The increase in the temperature will definitely enhance the rate of respiration of the planktonic algae and thereby the energy stored will be used up and reduces the multiplication rate. Roy (1955) stated that in the

Hoogly estuary the low winter temperature never acts as a limiting factor for the phytoplankton production. According to Shetty *et al.* (1961) the abundance of the phytoplankton in the Hoogly area during the June –August was due to the relatively high temperature (30^0C). But as per the view of Steeman Nielsen and Jensen (1957), in the shallow region where the bottom is in direct contact with the overlying water, the indirect influence of temperature causes an enhancement in the regeneration process to some extent which reflects in the rate of the primary production. Seasonal variations of thermal structure in the Arabian Sea showed distinct bimodal variation (Corlborn, 1971) which is also reflected in phytoplankton production.

In the present study the temperature was high during the pre monsoon season along the nearshore and inshore areas and doesn't show any direct relation with the productivity. Along the inshore areas the temperature showed the highest values during the premonsoon period as in the case of the estuary, but the values are higher, and showed no direct relation with the productivity except in Cochin area. In both these areas the range of temperature variation was low.

According to Quazim *et al.* (1972), blooming of the phytoplankton was during the low salinity along the Cochin

backwater. This indicates that the phytoplankton utilize the water enrichment maximum during this season. Subrahmanian (1959) also pointed out that a phytoplankton bloom can be expected along the Calicut coast when there will be a fall in temperature and salinity. In the present study also due to the maximum utilization of the nutrient enrichment by the phytoplankton, especially by the diatoms there was an abundance of the same during the monsoon. The salinity was low both along the marine and estuarine area during the monsoon period. This may be due to the dilution of water by rain fall. The productivity was also higher during the monsoon, showing direct relation with the salinity. Gopinathan (1981) reported that the salinity is one of the controlling factors of phytoplankton production in the estuarine systems. It was observed by him that the phytoplankton grow well in salinity of 15-20 ppt. In the inshore and in the oceanic environment also sudden fall in the salinity during monsoon months associated with high nutrient enrichment favour the phytoplankton production.

From observations made in various estuarine systems of Indian seas, it is seen that the "Biological spring" falls during the monsoon months, when the phytoplankton peaks coincide with low salinity and temperature associated with high concentrations of nutrients.

Quazim *et al.* (1972) have indicated that in the estuarine area of Cochin, the direct correlation of the phytoplankton production with low salinity could be an adaptation by the planktonic algae to utilize the available nutrients. Moreover, the distribution of phytoplankton standing crop in the different estuaries vary from place to place and time to time as a result of the water masses being constantly renewed by inflow of freshwater from the rivers, land run off and seawater from inshore area.

According to Sankaranarayanan and Quazim (1969) and Gopinathan *et al.* (1974), the instantaneous concentration of nutrients in the Cochinbackwaters has no significant role in the production of phytoplankton. In some cases inverse relationship were also seen. According to Selvaraj *et al.* (2003) nearshore waters are enriched with sufficient quantities of water throughout the year, and nutrients never acts as limiting factor for phytoplankton production. Higher values of nutrients were reported by them along the Cochin estuary.

The regression analysis proved that the nearshore and inshore areas of the three stations are independent of each other and the contributing parameters also vary from station to station. The probable reason for these variations is the effect of dynamic nature of the back water and also sampling method. Another reason

for these variations may be the influence of marine condition and influence of fresh water discharge which result in the process of mixing. The inverse relationship between the factor and diatom indicates that these variables have been used proportionately for the building up of diatom cells.

Chapter 3

3. Optimum nutritional requirement for the growth of *Chaetoceros calcitrans*

3.1 Introduction

As diatoms are known to be a good source of food for marine invertebrates, supplying both energy and nutrients in the form of protein, carbohydrates and lipids (Whyte, 1987) there is an increased trend of culturing them in mass scale. Research on mass culture of diatom has been carried out in many parts of the world for the past fifty years. The larvae of prawns and fishes prefer diatoms as basic food. The success of any hatchery operations depends mainly on providing the required food species of micro algae suitable for the larvae. Even after the two decades of research on the formulation of the micro diets to replace live food in larviculture, there is limited success (Watanabe and Ackman, 1974). Usage of diatoms as live feed depends mainly on the nutritional quality as well as their tolerance to temperature, salinity and light condition especially while maintaining stock culture, indoor and outdoor mass culture system. Eventhough alternate feeds are available in market, being costly; the micro algal culture is most economical live feed in hatcheries.

Diatom culture was also introduced to study the effects of various elements on the culture e.g. *Ditylum brightwelli* (Steel, 1965). The correlation between Cu toxicity and salinity was studied using *Skeletonema costatum* (Mandelli, 1969). The effect of elements especially vitamins on the culture of diatoms namely *Chaetoceros* and *Skeletonema* were done by Gopinathan (1981). Silicon as growth limiting nutrient for diatom has been established by silicon enrichment experiments of Schelke and Stoemer (1971), Gloosechenko and Alvis (1973) and Schelske (1984).

There are many culture medium for diatom culture such as Erd-Schriber's (Schriber, 1925) and Miquel's (Miquel, 1890) Walne's or Coway (Walne, 1974) etc. These media incorporate trace metals and several inorganic and organic salts. Although most algae are photo autotropic and can grow in purely inorganic medium many require organic compounds, the requirement of which may be either absolute or stimulatory. There are some organic and inorganic materials present in the seawater, which may be sufficient for their initial development. Micro algae perform much diversified and vital functions in an aquatic ecosystem like incorporation of solar energy into biomass, production of oxygen by photosynthesis that get dissolved into water, cycling and mineralization of chemical elements and as the food source for

herbaceous and omnivorous animals. When they die they sink to the bottom where their chemical constituents are transformed, solubilised and recycled into the water. These functions depend on phytoplankton population dynamics which in turn is determined by the behavior of individual species. These functions depend on phytoplankton population dynamics which in turn is determined by the behavior of individual species. The latter can be understood only by the laboratory studies in unialgal cultures.

Popularization and commercial application of photosynthetic biomass production systems like cultivation of algae are more relevant now than ever before in the international context of energy shortage, water disposal problems, environmental protection, alternative food additives and cheaper sources of feed proteins (Venkataraman, 1990). Production of microalgae for more varied and newer applications like aquacultural practices has come of age. Since the nutritional needs of algae differ with species, a large scale cultivation of any economically important algal species demands a thorough knowledge of its nutritional requirements.

There have been very few attempts in the past to study the nutritional requirements of microalgae under laboratory conditions. In this account an attempt was made to the optimum concentration of culture media required on suitable period of culture of

Chaetoceros calcitrans under controlled conditions so as to achieve a viable and economical method of culture. The main objective of the study was primarily to determine the effect of hydrological parameters especially nutrients which was supplied externally to the seawater while culturing the diatom under laboratory conditions, and to determine its optimum concentration for ideal growth of the culture, in such a condition of increased rate of pollution due to the industrialization and others.

3.2 Material and Methods

The material for this investigation was the culture of *Chaetoceros calcitrans,* which is widely used in aquacultural practices as live feed. The diatoms were grown in enriched seawater. Seawater collected from offshore of Cochin was transported to marine hatchery complex of CMFRI and kept 2-3 days in a settlement tank. Further, it was chlorinated by active chlorine and dechlorinated by aeration. Chlorinated seawater was filtered through absorbent cotton and boiled in 5 litre flask at 100^0C, cooled and kept in the culture room before further process. The culture room was being a controlled environment of temperature 25^0C, fluorescent light was provided from four ft. tube light above the culture flask and photoperiod was maintained in 16:8 hours of light and dark period.

Plate 1. Experimental setup

Flasks arranged in racks

Enlarged view

The experiments were conducted to determine the growth of *Chaetoceros calicitrans* in different concentration of enrichment. The experiment was conducted for a period of one month. In the first concept the enrichment was made by Walne's (1974) medium at different concentration such as 25%, 50%, 75% and 100% added on different days of culture. The 100% enrichment was taken as control. Duplicate samples were taken for all the treatments. The stock was maintained in the laboratory under controlled conditions of temperature (25°C), salinity (30 ppt) and the photo period of 16: 8 hours total darkness. An initial inoculum of $15x10^{6}$ cells/ml of culture was added to all the flasks.

3.2.1 Culture conditions

One litre of sterilized water was put in each 34 flasks containing different concentration of Walne's medium constituting 25%, 50%, 75% and 100% of enrichment. Further enrichment was given on initial, 5th, 10th and 15 days of culture period. All the 34 flasks were inoculated with 10 ml of stock culture of *Chaetoceros* having concentration of $14x10^{6}$ cells/ml. Two flasks without any addition of enrichment were also kept for observing the growth without providing additional enrichment.

In each treatment the medium is added at every 5 days interval. Each treatment group is taken for growth study

immediately after adding the medium, in a spectrophotometer at λ 430 nm. The readings of all treatments were taken in alternate days also. All the cultures were illuminated with light of fluorescent tubes.

Aeration was not provided to the cultures; instead cultures were shaken manually to give three to four rotations every now and then to keep them in uniform suspension. Growth study was carried out by three methods. (1) by measuring the cell count using haemocytometer (2) by measuring the growth by percentage of transmittance (3) by estimating the chlorophyll *a* content by spectrophotometry.

3.2.2 Measurement through cell count

The cells were counted under the microscope at 100x using a calibrated haemocytometer and expressed in number of cells/ml.

3.2.3 Measurement through percentage of transmittance

Cultures were taken for growth study at every alternate day using Genesys spectrophotometer. The transmittance rate was measured at 430, 530 and 678 nm with reference to the seawater.

3.2.4 Measurement through estimation of chlorophyll *a* content

Quantity of chlorophyll *a* is also used as an index of physiological activity. The concentration of chlorophyll *a* was

estimated by spectrophotometric analysis of acetone extracts (Strickland and Parsons, 1968).

A known volume of culture was filtered through filter paper; the pigments were extracted by adding 10 ml of 90% acetone to each residue. The extraction was carried out at low temperature for 20 hrs. The extracts were centrifuged and the supernatant was measured by Genesys spectrophotometer at 630, 645 and 663nm.

3.2.5 Statistical analysis

Data were presented as mean ± standard deviation and analyzed using two way analysis of variance (ANOVA). When a significant deviation was found, the mean values were tested fro the significant ($P<0.01$) by Duncan's multiple range test (Duncan, 1955). Statistical analysis was performed using the SPSS 10.00 version for Windows and results were tested for significance at 1% level.

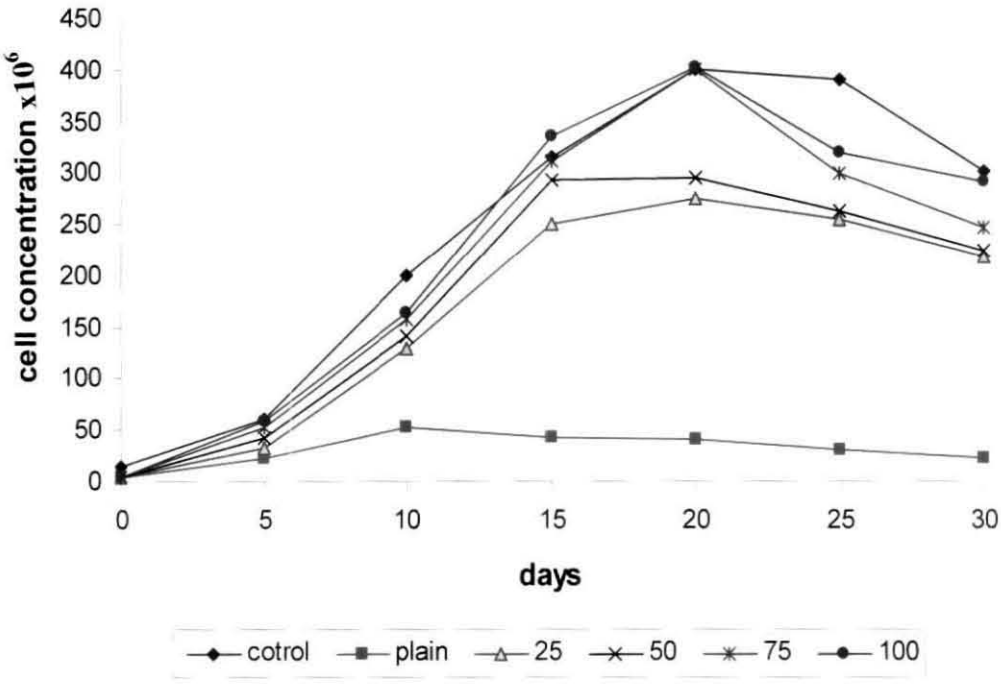
3.3 Results

3.3.1 Growth study by estimating the cell concentration

In the flask with 100% enrichment treated as control of the experiment indicated active transmittance reading up to 20^{th} day, after that the transmittance rate reading indicated declination in the growth rate. Minimum value indicating maximum growth was obtained in the 20^{th} day (36%) with reference to sterilized seawater.

Upon adding the enrichment on the initial day it was observed that there was not much difference in the cell concentration up to 5th day. On the 10th day the cell concentration was more prominent in the 100% concentration followed by the 75, 50 and 25% containing 168, 159, 142 and130x10^{6} cells/ml respectively. In the 100% enrichment the growth was found to be 19% less than the control. There was not much difference between the treatments. On the 15th day also the higher concentration was observed for the 100%, followed by the75, 50 and 25%. Not much difference was observed between the 100, 75 and 50 %, but the 25% showed a decline of growth by 16% from the 50%. On the 20th day it was 276, 296, 393 and 400x10^{6} cells/ml for 25, 50, 75 and 100% respectively. On the 25th day wide variation was observed between the ^{control} and 100% concentration. But there was not much difference between the 25 and 50% concentrations, and between the 75 and 100% concentration. The difference between 75 and 50% was11.5% on the 30th day at the same time it was almost same for 100% and control (Fig. 3.1).

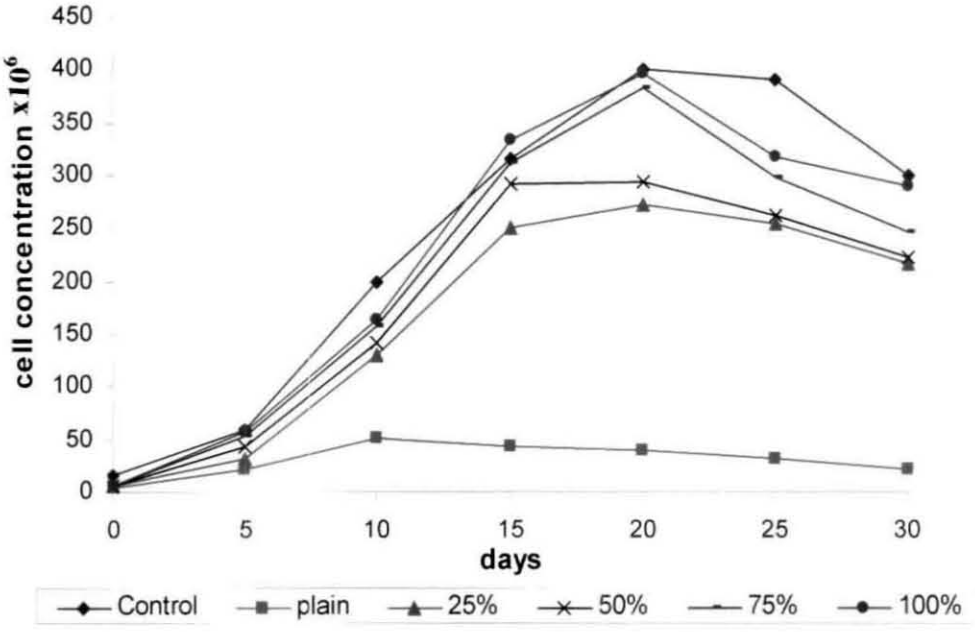
Fig 3.1 *Cell concentration on initial day enrichment*



Enrichment on the 5th day also showed a similar growth pattern up to 5th day. The growth pattern and rate was almost similar to that on the initial day. From 10th day onwards the growth increased with increase of enrichment showing a growth rate of 130,142, 157 and 163x10^6 cells/ml for 25-100% concentrations respectively. Control has a concentration of 200x10^6 cells/ml. There was a difference of 22.6% between the control and the 100% concentration. On the 15th day the control has 315x10^6 cells/ml while that for 25-100% was 250, 290, 318 and 330x10^6 cells/ml respectively. On the 20th day also more prominent growth was observed for the 100% followed by the 75, 50 and 25%, (330, 318,

290 and $250x10^6$ cell/ml respectively). Control has $400x10^6$ cells/ml; there was difference of 3.7 % between the 100 and 75%. On the 25th day wide difference in the cell concentration was observed between the 100% and the control (17.6%), control has high value, then between the 75 and 50% there was a difference of 10.7%. On the 30th day there was reduction in the cell concentration indicating a declination phase. The observed values were 224, 223, 247 and $294x10^6$ cells/ml for 25-100% concentrations. Control has $300x10^6$ cells/ml. The difference between 100 and 75% was 19% while that for the 50 and 75 was 10.7%. The acceleration phase was from 5-10th day. The rate of acceleration on the 5-10th day for the control was 23.35 while all the treatment groups showed more than 2 fold increase. From 10-15th day it was 57.5% for control, which was almost similar for the 100 and 75% and least for the 25%. On the 25th day the highest declination rate was observed for the treatments than the control (Fig. 3.2).

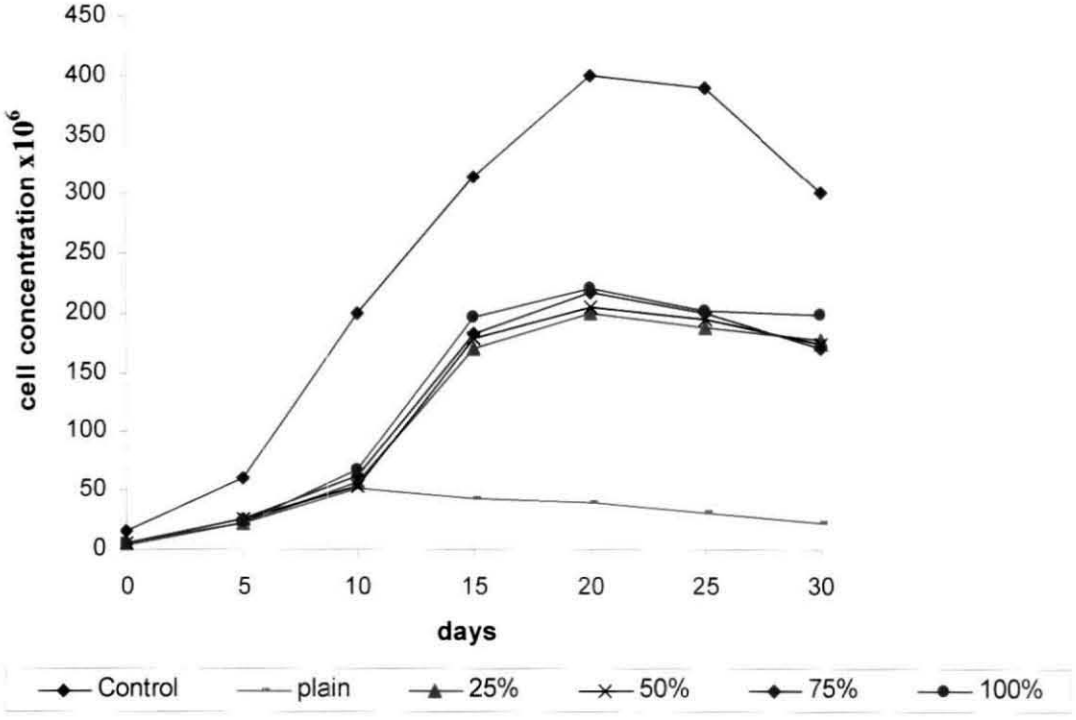
Fig. 3.2. Cell concentration on 5th day enrichment



Enrichment on the 10th day showed not much difference for all concentration upto 10th day. But wide variation from control was observed from 5th day onwards. Compared to the control there was much reduction in the cell concentration for all the treatment groups. Among the four treatment groups the higher concentration was noted for the 100%, followed by the 75, 50 and 25% respectively. On 5th day the control showed more or less 2 fold increase from the treatment groups. On 10th day it was three fold increase for control. On 15th day 1.5 fold increase was there for the control over the 100% concentration, while the 25, 50 and 75% has almost same cell concentration. On 20th day the maximum cell concentration was observed for the control and the treatments. The

75 and 100% concentration have same cell concentration, at the same time 50 and 25% have the same concentration. The difference between the 50 and 75% was 6.34%, while that for 100% and control was 44.75%. Almost same reading was observed for the 25th day's concentration. In short the values of cell concentration are much lesser than that on the 5th day's enrichment. (Fig. 3.3)

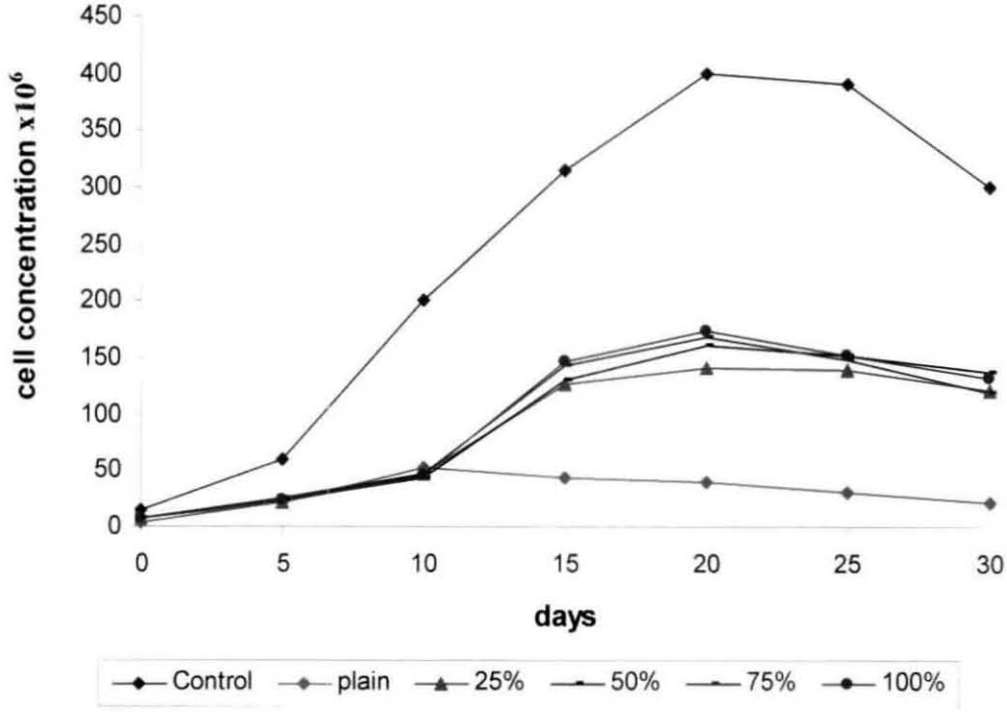
Fig. 3.3 ***Cell concentration on 10th day enrichment***



Addition of the medium on the 15th day indicates not much difference between the treatments up to the 10th day. On the 15th day the 25 and 50% showed not much difference between them. The values were 127, 130, 142 and 147 for 25-100%

concentration respectively. Between 50% and 75% there was a difference of 9.2%. On the 20th day maximum value was obtained for all. But the 25% showed the lower cell concentration, indicating that there was increase in cell concentration with increase in the media concentration. The values on 20th day were 200, 205, 218 and 221 for 25-100% concentration. On the 20th day the control was 131.1% higher over the 100% concentration indicating that the addition on the 15th day did not bring much increase in the cell concentration (Fig. 3.4).

Fig.3.4 ***Cell concentration on 15th day enrichment***



The ANOVA indicates that the cell concentration was significantly influenced by the day in which the enrichment was

provided concentration of medium and the age of culture. The DMRT indicated that the control has significantly (P<0.01) higher concentration of cell than the other. Among the treatment groups the 100% showed significant cell concentration followed by the 75, 50 and 25%. It was also revealed that on 20th day the cell concentration is significantly higher over the other and addition of medium on the 15th day showed significantly lower concentration than the others (Table3.1).

Table 3.1 DMRT for cell concentration

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	3238921.950	14	231351.568	34.669	.000
Intercept	7278087.355	1	7278087.355	1090.661	.000
Day of Enrichment	281920.225	3	93973.408	14.082	.000
Day of observation	2517848.956	6	419641.493	62.886	.000
Concentration	424807.115	5	84961.423	12.732	.000
Error	2135391.704	320	6673.099		

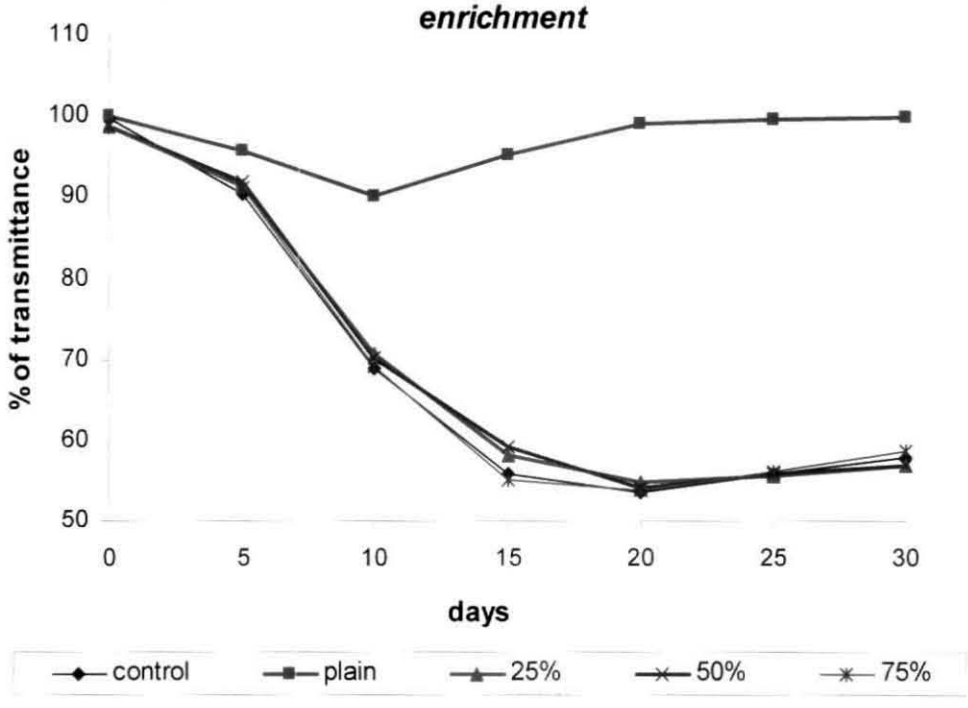
Significant at (P<0.01)

3.3.2 Growth study by estimating the percentage of transmittance

Enrichment of seawater on the initial day period did not showed wide variation of growth among treatments of 25. 50 and 75%. Where as there was a named increase in growth in the 100% enrichment, which was very prominent from 10th to 20th day of

growth. On 25th and 30th day there was declination indicated by increased transmittance values. The culture without enrichment showed growth only up to 10th day and there after declination. For all the concentration there was active phase from 5-10th day. Then from 15th day onwards only slow increase was noted and remains unchanged after the 20th day corresponding to the death phase of the growth. [Fig. 3.5].

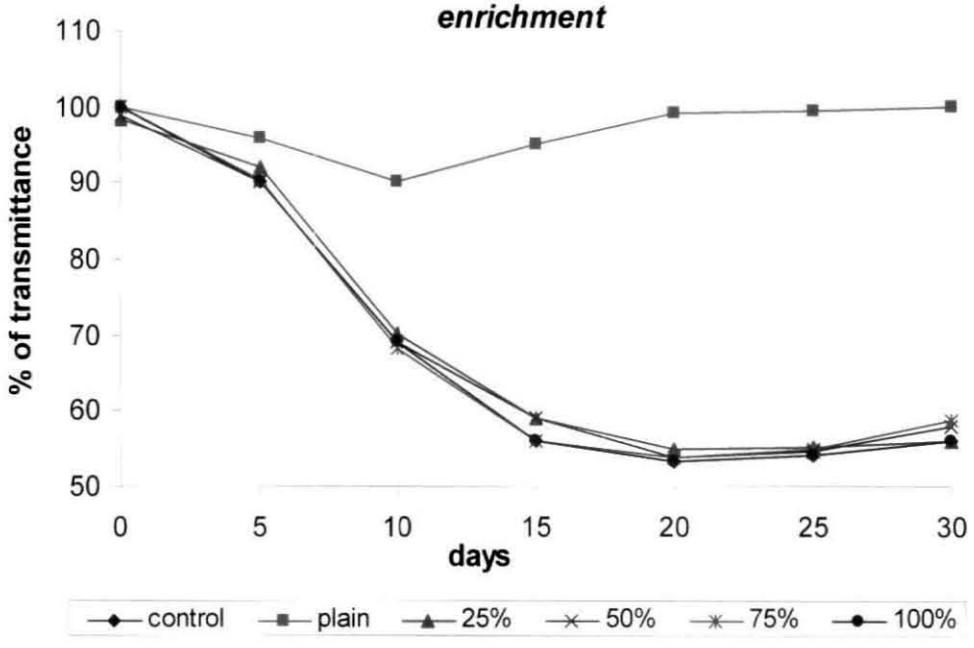
Fig. 3.5 ***Percentage of transmittance on initial day's enrichment***



Addition of enrichment on 5th day showed a pattern of growth which was similar to that of initial day's enrichment. Here the group without enrichment showed growth up to 10th day followed by decline phase. For all other treatment groups the growth rate was similar without much difference between them

throughout the culture period and the difference between the treatment and control was also negligible even on the exponential day constituting transmittance of 54.9, 53.9, 53.73 and 53.2% respectively for concentration ranging from 25-100%. The value of the control was 53.6%. In the decline phase also almost same value were observed for all the concentrations. The acceleration phase was between 5-10th day (Fig. 3.6).

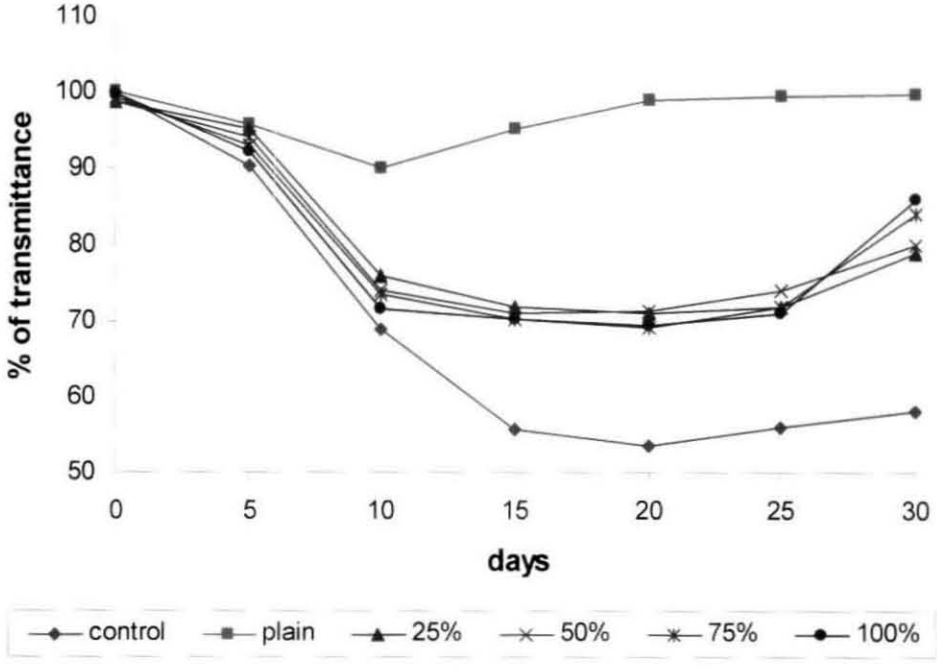
Fig 3.6 *Percentage of transmittance on 5th day enrichment*



The 10th day enrichment showed similar growth rate up to 5th day. But from 10th day onwards slight difference in growth was noted in which the lower transmittance was for the 100% followed by 75, 50 and 25%. The values were 76, 74, 73.4 and 71.6% for 25-100% concentration. The value for control was 68.9%. The

control showed an increase of 2.7% over the 100% concentration. While that for 100% over the 25% concentration was 17.1%. On 15th day also almost similar values were noted for the concentration ranging from 25-100%. On 20th day the maximum value was observed for all treatment groups. There was wide variation between the control and 100% (22.6%). The declination phase was from 25th day. The acceleration rate was higher for the control than the treatments (Fig. 3.7).

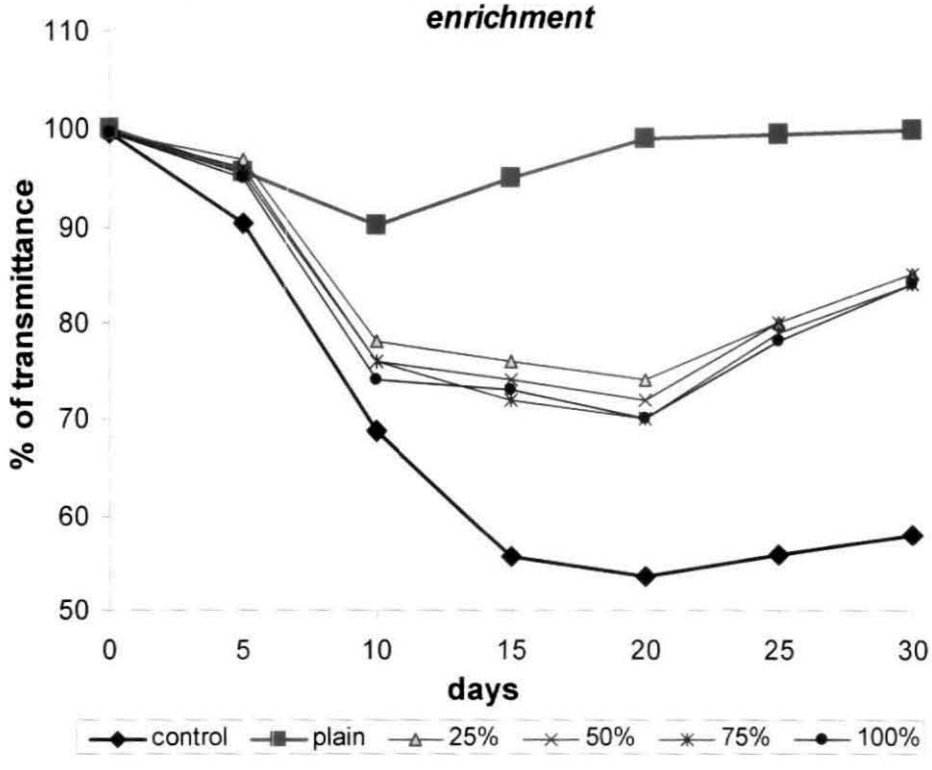
Fig. 3.7 *Percentage of transmittance on 10th day enrichment*



The enrichment on 15th day showed a transmittance almost similar up to 10th day with a decrease of 9.1% from the control. On 15th day also there was not much difference between the treatment groups 76, 74, 72 and 72 for 25-100% concentrations, while that

for control was 55.8 showing a high rate of reduction from the control. The 100% showed 16.4% lesser growth rate than that of the control. On 20th day minimum value of transmittance was noted for all 74, 72, 70 and 69.8 for 25-100% concentrations with more reduction in growth for 100% from the control (16.2%). The acceleration phase was from 5th day onwards. The rate of acceleration was higher for the control than the treatments. The declination phase was noted from 25th day onwards and the rate of declination was lower for the control than the treatments (Fig. 3.8).

Fig. 3.8 ***Percentage of transmittance on 15th day enrichment***



The three way ANOVA indicated that the transmittance was significantly affected by the concentration of medium, the day

on which the enrichment was provided and the age of culture. From the DMRT it was revealed that the 100% concentration produce significantly (P>0.01) lower values of transmittance indicating higher growth over the 25% concentration, at the same time enrichment on 15th day produce significantly higher transmittance indicating lower growth rate than the initial day. It was also clear that transmittance on 20th day was significantly lower compared to that on the initial day (Table 3.2).

Table 3.2 DMRT for transmittance

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	125715.080	14	8979.649	60.835	.000
Intercept	1736840.911	1	1736840.911	11766.710	.000
Day of Enrichment	2565.585	3	855.195	5.794	.001
Day of observation	95250.263	6	15875.044	107.550	.000
Concentration	27656.431	5	5531.286	37.473	.000
Error	47234.025	320	147.606		

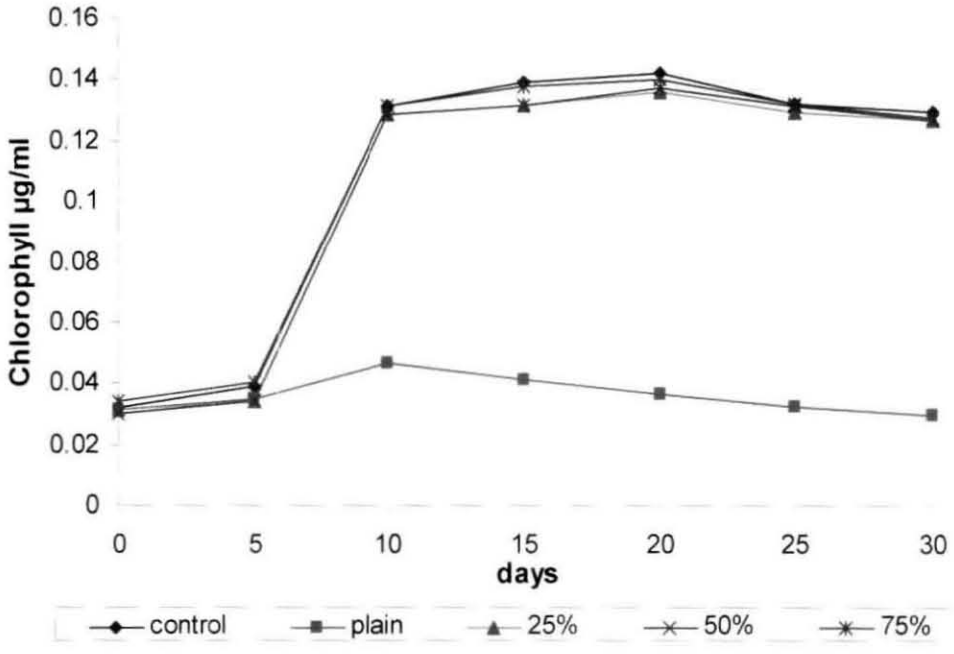
Significant at (P<0.01) level.

3.3.3 Growth study by estimating chlorophyll *a* content

Upon adding the enrichment on the initial day there was not much difference in the chlorophyll concentration among the treatments of 25, 50, 75 and 100% (control) concentrations, which was varied from 0.034 to 0.039 µg/ml. The 25% concentration has a chlorophyll content which is 12.8% lesser than that of the 100%

which is the control. Same pattern was noted up to the 30th day. Maximum chlorophyll values were observed on 20th day, but it was increased with increase in concentration constituting chlorophyll values of 0.135, 0.136, 0.139 and 0.141µg/ml for 25-100% concentration. Here not much difference was noted between the chlorophyll values of the four concentrations. On the 25th and 30th day also not much difference was noted between the chlorophyll values of all the groups. The lag phase was from the 0-5th day and active growth phase from 5-10th day, and then there was slow and steady increase in the chlorophyll value, followed by a declination from 25th day onwards. The rate of acceleration was higher for the 100% and least for 25%. On the 30th day not much difference was noted for the chlorophyll values of the four concentrations, 0.128, 0.130, 0.131 and 0.131µg/ml for 25-100% concentrations respectively (Fig. 3.9).

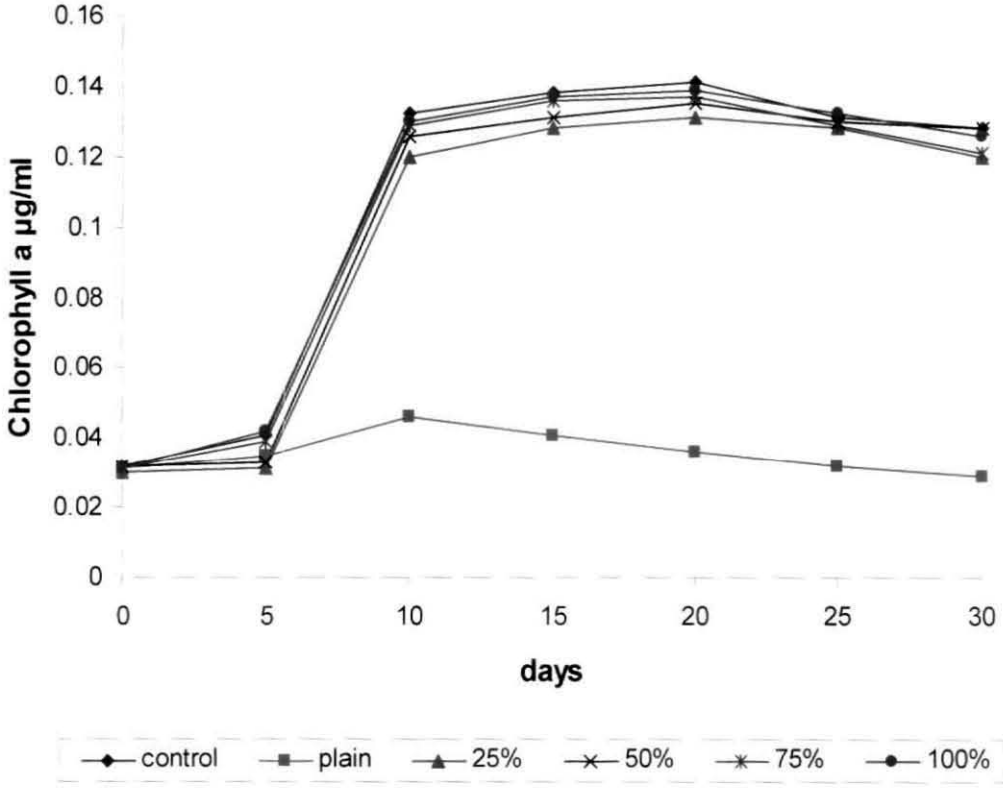
Fig. 3.9. *Chlorophyll a on Initial day's enrichment*



Providing enrichment on 5th day almost similar values of the initial day's enrichment was observed. Here also not much variation up to 5th day was noted. On 5th day the values were 0.031, 0.333, 0.039 and 0.042 µg/ml for the 25-100% concentrations respectively. Here the 100% has an increase of 7.7% over the 75%, at the same time 75% showed an increase of 18.1% over the 50%, and the 25% concentration was lesser than the 50% by 6.4%. On the 10th day there was marked difference (5%) between the 25 and 50% concentration was observed, 0.13, 0.129, 0.126and 0.120 for 100-25% respectively. The same pattern was for 15th and 20th day. The maximum value of chlorophyll *a* was observed on 20th day 0.131, 0.135, 0.137 and 0.139 µg/ml for

25, 50, 75 and 100% concentration respectively. There after, there was declination from 25th day onwards. Here also the lag phase was from initial to 5th day followed by an active growth phase from 10-15th day, then steady increase and declination (Fig. 3.10).

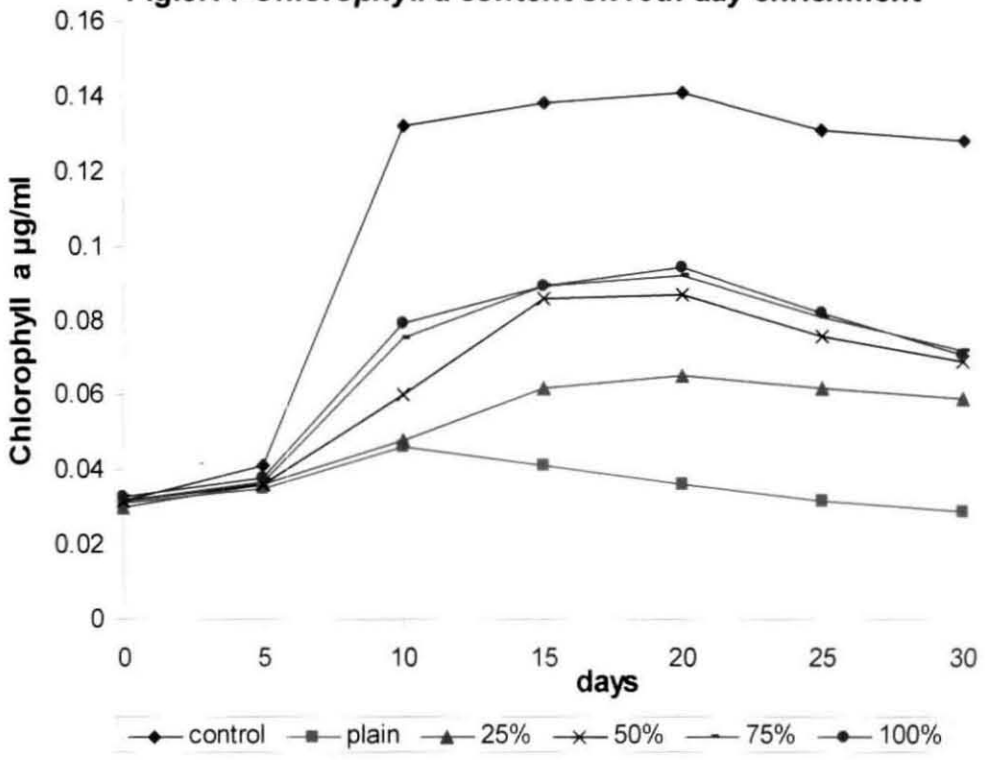
Fig. 3.10 *Chlorophyll a content on 5th day enrichment*



The chlorophyll *a* values on adding the enrichment on 10th day showed much reduction than that on 5th day. There was not much deviation up to the 5th day. In all the following days marked increase was noted for all concentration, and the increase was higher for the 100% concentration than any other treatments. The

culture without enrichment showed active growth up to the 10th day then declination was observed. On 10th day the value of 100% concentration was 67% lesser than that of control. The 75% concentration was 25% greater than the 50%. Between the 50 and 25% there was a difference of 25%. The values for chlorophyll *a* on 10th day were 0.079, 0.075, 0.061 and 0.48 µg/ml for 10-25% concentration, while that for control was 0.132 µg/ml. On the 15th day the 50, 75 and 100% concentration showed almost the same reading without much difference, 0.086, 0.089 and 0.089 for 50-100% respectively, which was nearly 55% les than the control. For the 25% concentration a value of 0.062 was observed which was 38% lesser than the 100% concentration. In that group in which no enrichment was added there were lower chlorophyll *a* values than the 25% concentration. On the 20th day same pattern was observed but a difference of 33.8% was there between the 25 and 50% concentration. The control showed an acceleration phase from 5-10th day (221.9%) increase. Then there was lower rate of increase from 10-15th day (4.5%) and from 15-20th day (2.2%) then declination was observed. The rate of acceleration for 100 and 75% is lesser than that of the control (102%) while that for 50 and 25% again less (33.3 and 66.6 respectively) (Fig. 3.11).

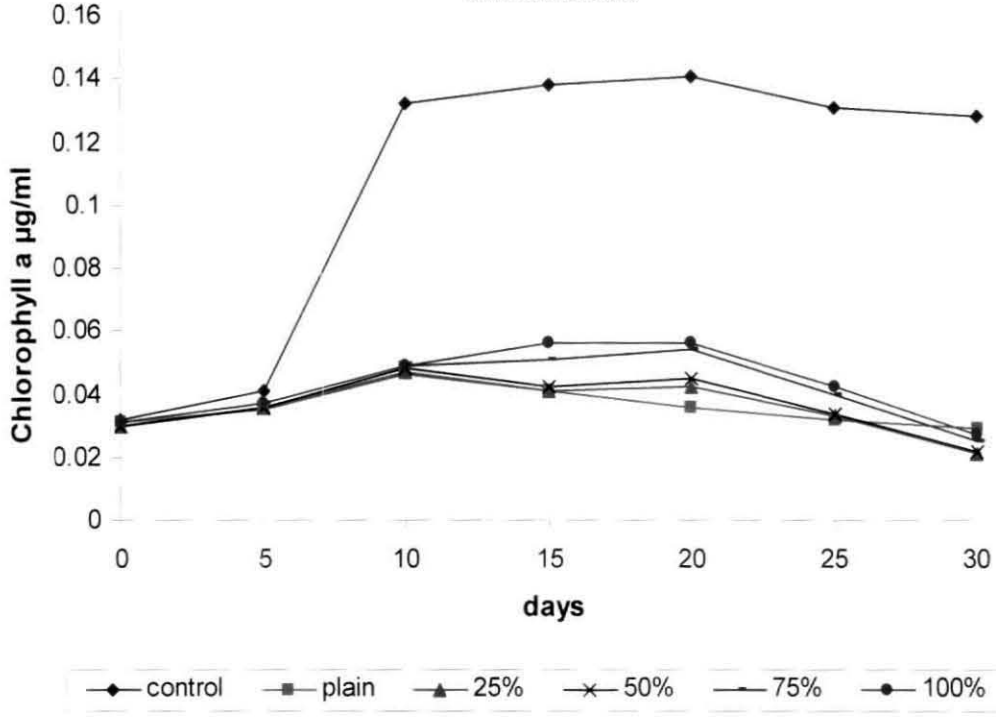
Fig.3.11-*Chlorophyll a content on10th day enrichment*



Upon adding the different concentration of enrichment on 15th day no difference was observed up to the 10th day. On 15th and 20th day growth was more pronounced in the 100% concentration followed by the 75, 50 and 25%. On 15th day the 25 and 50% concentration has almost same values 0.041 and 0.042 µg/ml respectively, while 75 and 100% concentration showed similar values of 0.051 and 0.056 µg/ml respectively. Much difference was observed between the 50 and 75% concentration on 15th and 20th day (21.4 and 20%). From the 25th day the growth was declined. In each day observation there was wide variation between the control and different treatments. Eventhough the

value for control was higher among the treatment; it was lower in comparison with the control (Fig. 3.12).

Fig.3.12 ***Chlorophyll a content on 15th day enrichment***



The three way ANOVA indicated that there was significant relation between the chlorophyll value and the day on which the enrichment was provided, the age of culture and the concentration of the medium. From the DMRT it was clear that the addition of medium on 15th day has significantly less effect ($P>0.01$) on the chlorophyll *a* value than the initial day, and the chlorophyll *a* value on the 20th day is significantly higher than that on initial day. It was also observed that the 100% concentration provides

significantly higher values of chlorophyll *a* than the 25% (Table 3.3).

Table 3.3 DMRT for chlorophyll *a*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.276	14	1.948	9.850	.000
Intercept	45.718	1	45.718	231.145	.000
Day of Enrichment	7.124	3	2.375	12.006	.000
Day of observation	17.277	6	2.880	14.558	.000
Concentration	2.832	5	.566	2.863	.015
Error	63.293	320	.198		

3.4 Discussion

The physical, chemical and biological factors of the aquatic ecosystem affect the species diversity of algae. Here an experiment was conducted to study the effect of nutrient concentration on *Cheatoceros calcitrans* under laboratory conditions, in order to asses whether the nutrients present in seawater is sufficient for the entire growth of culture or addition is needed.

The environmental conditions under which microalgae grow greatly affect the biochemical composition and may alter the energetic and nutritional value of the organisms. Usage of micro algae as live feed depends mainly on the nutritional quality as well

as their tolerance to temperature, salinity and light conditions especially while maintaining as stock culture, indoor and outdoor mass culture systems. In natural seawater blooming may be due to the nutrient enrichment as a result of upwelling, land run off and man made sources etc. Nowadays increased rate of nutrients like NO_3, NO_2 and PO_4 was there in the seawater due to increased use of chemical fertilizers, the influx of water containing the residuals of fertilizers will cause the increased rate of these nutrients in the seawater. In addition to this, as a result of industrialization large amount of sewage were getting deposited in the seawater. Actually we are using the enriched seawater for culture experiment, by adding further chemicals. This experiment was conducted to study whether there is any need of additional amount of chemical enrichment for growth of microalgae. Gopinathan (1981) reported that the soluble fraction of hydro carbons at low concentration seem to enhance the rate of photosynthesis of microalgae.

The potentialities of algae as source of food, feed, fodder and manure has been further established by the extensive research carried out during the past few decades. The economic utilization of algae necessitates the development of techniques for axenic culturing of these organisms in large scale. According to Venkataraman (1960a) and Gopinathan (1986) no single medium

can be said as the best one. Since the nutritional requirements of algae vary with species, the successful and long term culturing of any algal species demands a thorough understanding of its nutritional requirements which can be studied under controlled laboratory conditions using unialgal cultures. The works of Ammini Joseph (1983) on *Isochrysis* and *Tetraselmis* and that of Sathi (1992) on some phytoflagellates have shown that in higher concentrations of nutrients there was reduction in the chlorophyll *a* content than in the optimum. Varying concentrations of nutrients influence the productivity in culture system.

From the present study, it is clear that there was increase in the rate of growth along with increase in concentration in each enrichment except in plain. In plain the maximum growth was up to 10^{th} day. In all others it was up to 20^{th} day. The maximum growth was on 5^{th} day enrichment flask. In all others it was reduced. The maximum utilization was found on 5^{th} day. So we can conclude that up to 5^{th} day there is no need of enrichment because the system can utilize the nutrients of the seawater itself up to the 5^{th} day and we can save the chemicals up to the 5^{th} day.

In the cell concentration study also eventhough there is increase in cell concentration along with the increase in the medium concentration in every day's addition there is much more

increase in the 5th day's addition itself compared to that of 10th day and 15th day. This is due to the increased enrichment of seawater nowadays due to sewage and other effluents.

The chlorophyll *a* values of the 5th day's enrichment and initial day's enrichment showed similar growth pattern, indicating not much fluctuation upto the 5th day. As compared to the early days, the enrichment of seawater is very high in the south west coast of India due to the industrial effluents and the culture can survive upto the 5th day without any additional enrichment in the highly enriched seawater.

Form the present investigation it is evident that the increased rate of various nutrients in the seawater will definitely affect the growth of the *Cheatoceros calcitrans* under laboratory condition during the initial days of growth.

Chapter 4

4. Effect of trace elements and vitamins on the growth and biochemical composition of *Chaetoceros calcitrans*

4.1 Effect on growth

4.1.1 Introduction

An understanding of the mechanism of biological and chemical interaction among trace elements and planktonic organisms is the key to elucidate the role of trace elements in the ecology of oceans and role of the organisms in the geochemistry of metals. Most of the trace elements are present in living organisms in low concentration. According to Arnon (1950), an element is considered essential for an organism, when the organism can neither grow nor complete its life cycle in its absence or it cannot be replaced by any other element and has a direct influence on the metabolism of the organism. Earlier works performed on the mineral nutrition have revealed that the nutritional requirements of the algae are fairly similar to those for the complex phanerogams (Venkataraman, 1960). According to Ketchchum (1954) the requirements of these nutrients by the algae may be absolute, control, minimum or optimum. When an algae cannot grow and carry out its life processes in the absence of a

nutrient and that cannot replaced or substituted by any other, then the algae is said to have an absolute requirement for that element. The control requirement is the quantity of each nutrient contained in cells produced during active growth of a population, while no nutrient is limiting. The minimum requirement is the quantity of a nutrient in the cell when it is limiting the growth of the population, all other nutrients being present in excess. The concentration that permits the maximum growth and other metabolic processes is known as optimum concentration.

For the control growth and reproduction of organisms, micro nutrients or trace elements are necessary in at least very low concentration and these elements cannot be replaced by any other mineral factors. The micronutrients needed by various algal species are iron, manganese, Zinc, Copper, Molybdenum, Cobalt, Vanadium, Boron etc. (Most of these elements belonged to the class heavy metals).

Many of the trace elements are control constituents of aquatic organisms and are essential for their metabolism by having definite roles. Each of the basic elements either singly or in combination, along with the major environmental parameters can affect the biota as a whole and food chain in particular. Studies of De Filippis and Zeigler (1993) on the effect of sub lethal

concentration of zinc, cadmium and mercury have shown that these metals retarded the activity of four enzymes involved in the fixation of CO_2. Phytoplankton species vary in their tolerance to trace elements. Investigators like Gibson (1972), Ithack and Gopinathan (1995), Angadi *et al.* (1996), Graham *et al.* (1996) Knauer (1996) and Buttacharya *et al.* (2000) found out the tolerance limit of various phytoplankton to different trace elements like Cu, Zn, Pb, Ni, Mn and Cd. Differential response of marine diatoms to trace metals has been studied by Tadros *et al.* (1990).

In spite of the importance of these trace elements in algal growth and metabolism, a little attention has been paid on them on the nutritional point of view (Lec *et al.*, 1994; Lin *et al.*, 1994; Chow *et al.*, 1998; Katiyar and Katiyar, 2000). Most of the reports available focus on the toxicological impacts of trace elements especially those belonging to the class heavy metals (Malanchuk and Greuleling, 1973; Maeda *et al.*, 1970; Ning – Zheng *et al.*, 1990; Angadi *et al.*, 1996; and Buttacharya *et al.*, 2000). They furnish meager information regarding the significant of trace elements in algal nutrients.

In addition to the above mentioned elements, certain organic compounds including vitamin B_1, B_{12} and Biotin are found to be essential for growth of some algal species in culture system.

Miquel (1890) and Schreiber (1927) were among the pioneers to initiate the culturing of micro algae. Filtered seawater with adequate amount of nitrate and phosphates was used as the basic growth medium for these organisms. Pringsheim (1949) summarized the history and production of Phytoplanlton culture. In 1950's Provasolie, Droop and others modified the culture medium by adding basic metals, chelating compounds and vitamins (Provasolie *et al.*, 1954; Provasolie, 1958 Droop, 1954, 1968). The mineral requirements of micro algae were further reviewed by Lewin (1962), Stein (1973), Steawart (1974), Walne (1974) and Ward and Parish (1982).

Since nutritional needs of algae differ with species, a large-scale cultivation of any economically important algal species demands a thorough knowledge of its nutritional requirements. Popularizations and commercial application of photosynthetic biomass production system like cultivation of algae are more relevant now than ever before in the international context of energy shortage, water disposal problems, environmental protection, alternative food additives and cheaper sources of feed protein (Venkataraman, 1990). Production of micro algae for more varied and new ever application like aquaculture practices has come of age.

There have been very few attempts in the past to study the nutritional requirements of micro algae under laboratory conditions. Huge volumes of literature are available regarding the response of algae to higher concentration of trace elements. But literature regarding their response to lower concentration or elimination of trace elements and vitamins are scanty. The present work was undertaken with an objective to elevate the influence of elimination of trace elements and vitamins on the growth and biochemical composition of *Chaetoceros calcitrans.*

4.1.2 Materials and Methods

The diatoms were grown in enriched seawater. Prior to preparation of culture medium, the seawater collected from offshore and stored in CMFRI hatchery was brought to the lab in carbouys. Further, seawater was filtered and sterilized and poured to conical flasks of 1 litre capacity. A total No of 16 flasks were used for the experiment. Then the flasks were divided in to 8 groups. Ist group containing 2 flasks in which control was taken. In the 2nd group no medium was added. In the third group medium without the trace element Cu was added, in the fourth group the trace element molybdenum (ammonium molybdate) was eliminated. While in the fifth and sixth groups Zn and Co were

excluded. The 7th and 8th group is without vitamin B_1 and B_{12} respectively.

Immediately after the transfer of sterilized seawater into the flasks, 10ml of stock culture of *Chaetoceros* with a concentration of $14x10^6$ cells/ml was added. Salinity of seawater used was ±30ppt. The culture medium used was Walne's (Walne, 1974). Then all the flasks were plugged with cotton and the cultures were illuminated with fluorescent tubes. Aeration was not provided to the cultures; instead cultures were shaken manually to give three to four rotations every now and then to keep them in uniform suspension. Settling was not noticed for a month, but later on developed tendency to settle down.

Each group is taken for growth study immediately after adding the medium, in a spectrophotometer at wavelength 430, 540 and 678 nm. The readings of all groups were taken in alternate days also.

4.1.2.1 Measurement through cell count

The cells were counted under the microscope at 100x using a calibrated haemocytometer and expressed in no. of cells/ml.

4.1.2.2 Measurement through percentage of transmittance

Cultures were taken for growth study at every alternate day using Genesys spectrophotometer. The transmittance rate was measured at 430, 530 and 678 nm with reference to the seawater.

4.1.2.3 Measurement by estimation of chlorophyll *a* content

Quantity of chlorophyll *a* is also used as an index of physiological activity. The concentration of chlorophyll *a* was estimated by spectrophotometric analysis of acetone extracts (Strickland and Parsons, 1968).

4.1.2.4 Statistical analysis

Data were presented as mean ± standard deviation and analyzed using two way analysis of variance (ANOVA). When a significant deviation was found, the mean values were tested for the significant ($P<0.01$) by Duncan's multiple range test (Duncan, 1955). Statistical analysis was performed using the SPSS 10.00 version for Windows and results were tested for significance at 1% level.

4.1.3 Results

4.1.3.1 Growth of *Chaetoceros calcitrans* in nutrients eliminated medium by cell concentration study

As in the case of previous experiments, in this experiment also cell concentration was studied on every 5th day for a period of one-month.

It was observed in the control that on the initial day the cell concentration was 14x10^6 cells/ml. Then there was an acceleration hase upto 5th day, and the by peak period was observed on 20th day (540x10^6 cells/ml). Then there was steady growth up to 25th day followed by retardation phase, constituting concentrations of 14, 70, 180, 397, 540, 508 and 503x 10^6 cells/ml respectively.

The concentration study in the copper eliminated culture showed the same growth pattern. On the exponential phase the concentration was 493x 10 ^{6} cells/ ml showing a decrease of 8.7% from the control, thereafter a decline phase of 437x10^{6}cells/ml, while that on the initial, 5th, 10th and 15th day was 12, 67,164 and 300x10^6 cells/ml, with a decrease of 14.28, 4.2, 8.8 and 24.4% respectively from the control.

In molybdenum eliminated cultures also similar growth pattern was observed. The peak value noted on 20th day with 497x10^6 cells/ml indicating a reduction of 4.96% from the control.

While that on initial, $5^{th,}$ 10^{th} and 15^{th} and decline phase was 12, 62, 140 and $342x10^6$ cells/ml showing a reduction of 14.2, 18.33, and 25.19% respectively from the control.

In zinc eliminated cultures also similar growth pattern was observed. The peak value observed on 20^{th} day with 497x106 cells/ml indicating a reduction of 7.96% from the control. While that on the initial, 5^{th}, 10^{th} and 15^{th} and declination phase was 12, 62, 140 and $342x10^6$ cells/ml showing a reduction of 14.2, 25.7, 22.22 13.85 and 9.4% respectively from the control.

The cell concentration study in the cobalt eliminated culture showed a similar pattern of growth with a peak period on 20^{th} day. But the concentration of cells on the peak period was $494x10^6$ cells/ml with a reduction of 8.51% from the control. The cell concentration on 5^{th}, 10^{th}, 15^{th} ,20^{th} and 25^{th} day was 68, 143, 344, 494, 419 $x10^6$ cells/ml with a decrease of 25.7, 20.5, 13.35, 8.51and 17.5% respectively from the control. The declination phase has 419 $x10^6$ cells/ml while that for control was 508 $x10^6$ cells/ml.

Vitamin B_1 eliminated medium showed gradual increase of cell concentration from initial day onwards with peak period on 20^{th} day as in other cases, 18, 69, 171, 350, 523 with a reduction of 28.6, 27, 5, 11.8, and 0.6% respectively from the control. But the

concentration of cells on exponential phase was $523x10^6$ cells/ml while that of control was $540x10^6$ cells/ml indicating a reduction of 0.6% from the control. Declination phase shows concentration of $502x10^6$ cells/ml while that of control was $508x10^6$cells/ml.

Vitamin B_{12} eliminated medium also showed the same results in the growth pattern with less fluctuations. But the concentration of cells on the exponential phase was $523x10^6$ cells/ml while that of control was $540x10^6$ cells/ml. Here the retardation rate in each day from the control is almost similar to that of the B_{12} eliminated culture. Declination phase has a cell concentration of $503x10^6$cells/ml (Fig 4.1).

From the ANOVA it is clear that the cell concentration was significantly ($P<0.01$) affected by the elimination of the trace elements and vitamins and the age of culture. DMRT indicate that the cell concentration of the control was significantly ($P<0.01$) higher over the plain, among the treatment groups the least value was shown by the plain followed by Zn, Mo, ,Co, Cu, B_1 and B_{12} respectively, at the same time all the treatment groups except the plain formed homogenous group (Table 4.1).

Fig. 4.1 *Cell concentration on nutrient elimination*

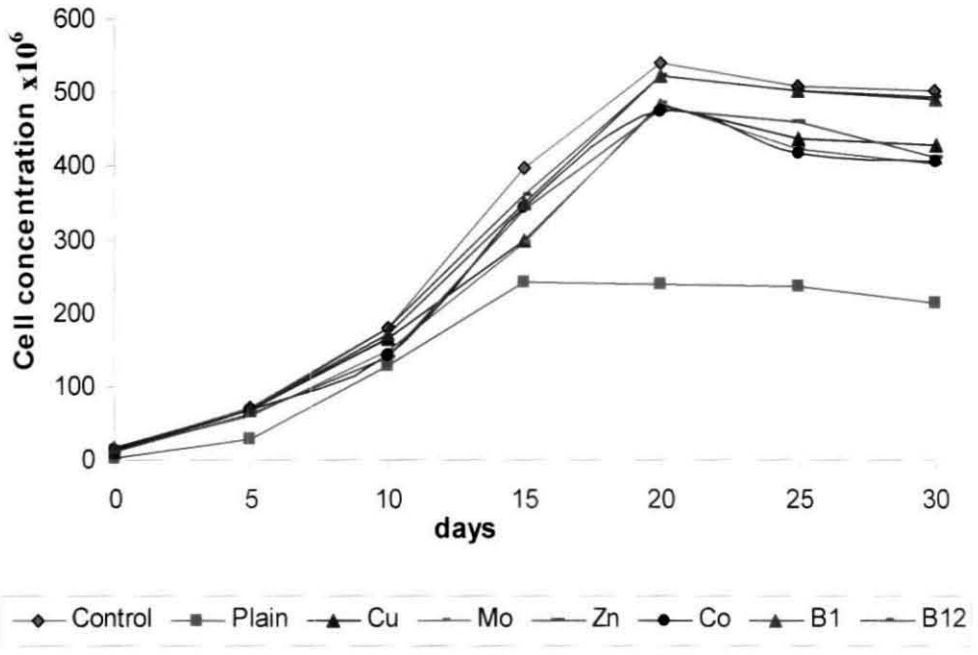


Table 4.1 Interaction of day and treatment on cell concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3569646.009	13	274588.155	83.001	.000
Intercept	7937977.580	1	7937977.580	2399.457	.000
Day of observation	3313309.232	6	552218.205	166.922	.000
Treatment	256336.777	7	36619.540	11.069	.000
Error	324207.411	98	3308.239		

Significant at ($P<0.01$) level.

4.1.3.2 Growth of *Chaetoceros calcitrans* in nutrients eliminated medium by transmittance study

A study on transmittance rate was also conducted and each set examined separately using Genesys Spectrophotometer, on every 5th day until the 30th day, at varying wave length of 430 nm, 540, and 678 nm.

In the control the minimum value of transmittance indicating maximum growth was observed on 20th day. From this result it was observed that the peak or exponential phase was on 20th day *i.e.*, 52.65. In the initial day maximum transmittance was there 99.8%, on 5th day it was 89.1, 10th day 67.1% and on 15th day 55.54%. From this it is clear that the growth showed a lag phase and then acceleration from 5th day, the exponential phase on the 20th day. There after declination phase noticed on 25th day onwards *i.e.*, 54.25 and on 30th day it was 62.15. Here it was clear that there is initial lag phase growth then gradual increase followed by exponential period thereafter declination as in every usual culture.

In copper (Cu) eliminated medium same pattern of growth was observed but the rate of transmittance was still higher on the 20th day. That was 54.5 while that of control was 52.65 indicating 3.5% decrease from the control. In the absence of Cu there was slight variation in the performance as compared to the control was noted on the exponential phase. This effect can be visible in every 5th days of transmittance rate *i.e.*, on 5th, 10th, 15th day the rate was 89.6, 67.3, 57.65 while that of control was 89.1, 65.1, 55.45 respectively, indicating a decrease of 0.56, 3.27 and 3.8% decrease in growth rate from control. On 25th day onwards there was declination 63.3 and 63.75 which was 16.68 and 2.5% decrease

from the control. Hence from these results it was also clear that there was reduction in rate of growth of the diatoms as compared to the control from the starting itself in the case of cell concentration on elimination of copper.

The elimination of molybdenum (Mo) also indicated similar effect on the transmittance rate of the culture. In Mo eliminated culture transmittance pattern is similar to those of control and the rate is somewhat same. The exponential period was observed on 20^{th} day as in the case of control. But the rate of transmittance showed only a slight difference from that of control. In this particular case it was 54.1% while that of control was 52.65% on 25^{th} day with a decrease in the growth rate by 2.68%. On 5^{th}, 10, 15 day the transmittance rate was 87.1, 62.1 and 58.1% respectively while that of control was 89.1, 65.1, and 55.45% showing a decrease of 2.24, 4.61 and 4.77% from the control. Declination phase was with a value of 68.4, and 66.65 while that of the control was 54.2 and 65.15 showing a decrease of 26.08 and 33.35% respectively from the control. From this result it is clear that the Mo elimination affects the culture growth moderately.

In zinc (Zn) eliminated culture, there was similar effect on the rate of transmittance. Exponential phase was on the 20^{th} day itself but, the rate was 56.0% which was 6.3% less than the control.

While that on the 5th, 10th and 15th day was 98.2, 61.4, and 54.35% respectively showing a reduction of 10.2, 5.68 and 2% from the control. Thereafter there was declination phase with 63.3% transmittance. From this result it was very clear that the Zn elimination results in the reduction of growth of the culture.

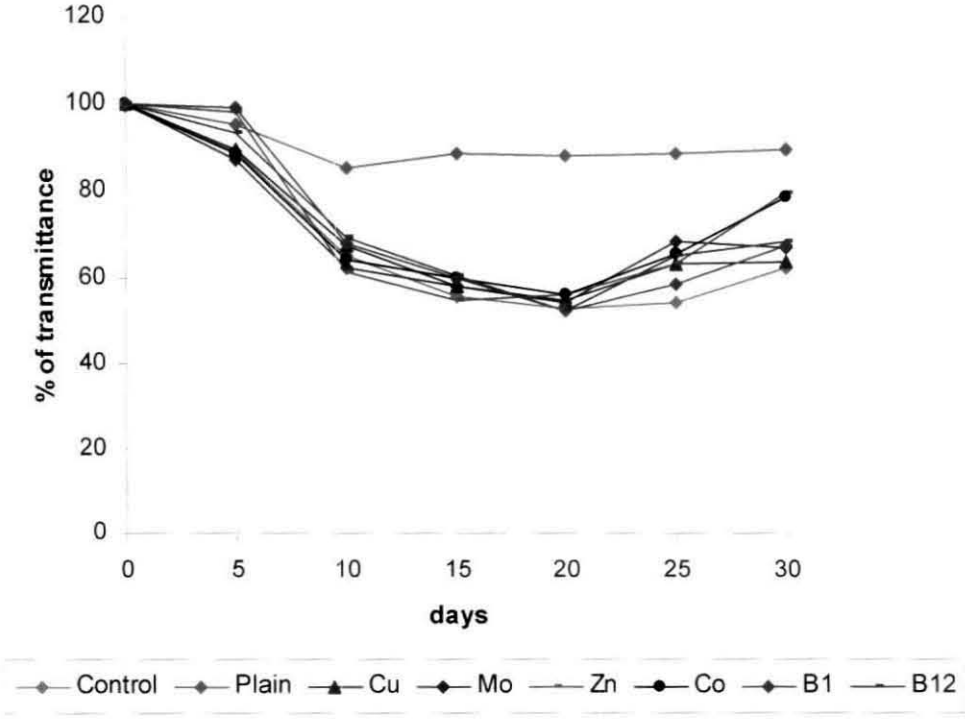
In the copper eliminated culture, the exponential day was on the 20th day itself the transmittance rate of which was 57.25% which was very high when compared to that of control *i.e.*, 52.65 % indicating a reduction in the growth by 8.7%. The 5th, 10th and 15th day transmittance was 99.9, 88.9, 64.2, and 59.7% showing a reduction in growth by 14.4, 0.22, 1.4, and 7.66% respectively. Here also there is an indication of reduction in normal growth rate of the culture in the absence of cobalt.

Vitamin B_1 elimination also indicates the exponential period on the 20th day as in all other cases. But the interesting factor which was quite different from the elimination of the other elements was that the transmittance value on this particular day was 52.25 which was very much nearer to the control *i.e.* 52.65 indicting same growth rate. The values on the zero, 5th, 10th and 15th day was 98.9, 68.0 and 62.8 respectively representing 10.6, 4.45and 13.25 % decrease from the control. This gives us a clear-

cut idea that elimination of B_1 affects the growth rate to some extend.

In vitamin B_{12} eliminated culture, the growth pattern was similar to that of the others. The transmittance rate value on the exponential phase was 52.35 which were almost similar to the control. The values on the 5th, 10th and 15th day were 93.5, 69.2 and 69.32 with a reduction in growth by 4.9, 6.29 and 25% respectively. There after there was declination. All these indicate that there is significant reduction in the growth rate in the absence of the trace elements and vitamins (Fig. 4.2).

Fig. 4.2 *Transmittance on nutrient elimination*



ANOVA indicates that the chlorophyll *a* value was significantly affected by the elimination of the trace elements, vitamins and aging of the culture. DMRT showed that the chlorophyll value of the control is significantly ($P<0.01$) higher over the plain and Zn, Mo, Co, Cu, B_{12} and B_1 eliminated treatments forming homogenous group. The value on the 20th day was highly significant over the others (Table 4.2).

Table 4.2 Interaction of treatment and day on rate of transmittance

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	28401.325	13	2184.717	72.562	.000
Intercept	617143.766	1	617143.766	20497.41	.000
Treatment	4561.378	7	651.625	21.643	.000
Days	23839.947	6	3973.325	131.967	.000
Error	2950.620	98	30.108		

Significant at ($P<0.01$) level.

4.1.3.3. Growth of *Chaetoceros calcitrans* in trace metal and vitamin eliminated medium by Chlorophyll *a* study

Conducted a chlorophyll *a* pigment study for a period of one month at an interval of every five days. There was a gradual increase from the initial day onwards. The maximum growth was observed on the 20th day and the chlorophyll *a* value was 1.527 µg/ml for the control. There after there was a stationary phase with

the chlorophyll value of 1.19 µg/ml and then declination phase initiated. The chlorophyll *a* values for the initial, 5th, 10^{th} and 15^{th} day were 0.045, 0.28, 0.99, and 1.33 µg/ml respectively. The same growth pattern was observed in all other cases.

In the copper eliminated culture the value on the exponential phase was 0.90 µg/ml and that on the stationary phase was 0.73µg/ml showing a decrease of 41.06 and 52.1% respectively. The values for the initial, 5^{th}, 10^{th} and 15^{th} day were 0.02, 0.18, 0.72, and 1.099 µg/ml respectively indicating a decrease of ranging between 18-55% from the control. These values indicate that there was reduction in the growth of the culture in the absence of copper.

In the absence of molybdenum (ammonium molybdate) the peak value was 1.16 with a reduction of 24.03% from control, and that for the initial, 5th, 10^{th} and 15^{th} day were 0.02, 0.09, 0.87, and 0.90 µg/ml respectively indicating that there is reduction in the absence of molybdenum in the growth of *Chaetoceros* by 55.5, 67.8, 12.12 and 66.3%.

Chlorophyll *a* values for the zinc eliminated cultures were 0.01, 0.22, 0.89, 1.22, 1.36 µg/ml up to the exponential day. Here also there was reduction in the growth rate in the absence of Zn by 77.77, 21.43, 10.10 and 8.2% respectively from the control indicating the influence of Zn on the growth of the culture.

Cobalt eliminated medium also indicate difference of chlorophyll *a* content in comparison with the control. The values were 0.037, 0.25, 0.71, 1.11, 1.29 µg/ml etc up to the 20th day. There was reduction in the growth rate in comparison with the control by 17.77, 10.7, 28.2, 16.5 and 15.5% indicating the influence of cobalt enrichment in the medium.

In the vitamin B_1 and B_{12} eliminated medium there was reduction in the chlorophyll *a* values. The values for the vitamin B_1 eliminated medium were 0.028, 0.17, 0.94, 1.22 and 1.38 µg/ml up to the 20^{th} day. There after declination phase with a value of 0.69 µg/ml indicating retardation by 41.6, 40.7, 5, 8.27 and 9.67% from the control. While that for the B_{12} eliminated medium showed the values of 0.029 µg/ml, 0.16 µg/ml, 0.94, 1.196 and 1.40 µg/ml upto the 20^{th} day showing almost same reduction rate from the control as that for the B_1. The declination phase showed a value of 0.69µg/ml (Fig. 4.3).

The two way ANOVA indicates that the chlorophyll *a* value was significantly affected by the elimination of trace elements, vitamins and different day of observation. From DMRT it was clear that the control was significantly ($P<0.01$) higher in chlorophyll *a* content than any other treatment group. Among treatment groups the maximum value of chlorophyll *a* was

indicated by vitamin B_{12}, followed by B_1, Mo, Co, Cu and Zn with least difference between them, at the same time forming homogenous group. It was also clear that the chlorophyll *a* value on the 20th day was significantly higher than that on the initial day. (Table 4.3).

Fig.4.3 *Chlorophyll a content on nurtrient elimination*

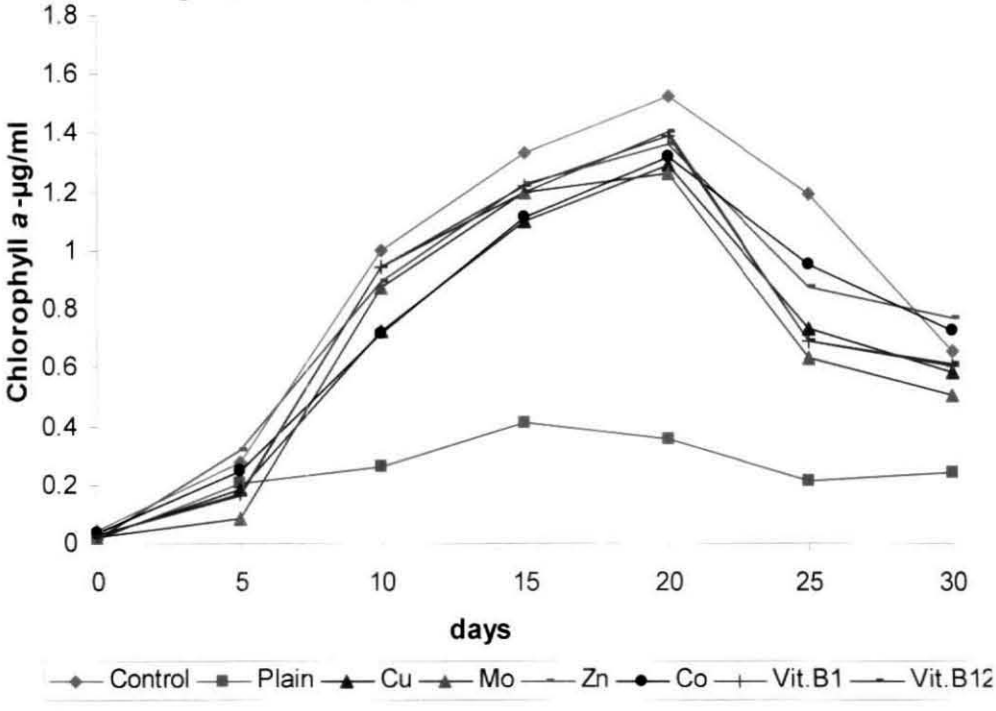


Table 4.3 Interaction of treatment and day on chlorophyll *a* content

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	20.767	13	1.597	54.861	.000
Intercept	52.803	1	52.803	1813.422	.000
Treatment	3.455	7	.494	16.952	.000
Day of observation	17.311	6	2.885	99.088	.000
Error	2.854	98	2.912E-02		

Significant at (P<0.01) level

4.1.4 Discussion

Plankton are very much sensitive to the environment in which they live and therefore any alteration in the environment will lead to the change in the plankton communities in terms of tolerance, abundance, diversity and dominance in the habitat. Since industrial revolution, the efforts of removing the pollutants from the natural environment have not been able to keep pace with the increasing amount of waste materials and growing population that further aggravates the situation. The elements like copper, zinc, cobalt, molybdenum etc have important biological functions in the aquatic organisms. The relatively short life time and larger surface area of phytoplankton community makes it very susceptible to changes in various concentrations and they are found to be limited by different heavy metals (Mandelli, 1969). The present study is to find out the effect of exclusion of trace elements and vitamins from the *Cheatoceros calcitrans* culture in the lab condition. Trace amounts of copper are essential for metabolic process of algae. Essentiality of copper as micro nutrient has been confirmed by a number of investigators (Green *et al.,* 1939; Walker, 1953; O' Kelley, 1974; Sorentine, 1979). Diatoms are least sensitive to copper toxicity (Sunda and Guillard, 1976). Srisudha (1989) reported that lower concentration of copper is essential for the early

growth phase of flagellates, but later higher concentration is needed for further growth. According to Price and Quigely (1960) the growth of *Euglena gracilis* in zinc limited culture was markedly higher in medium lacking copper.

Subba Rao (1981) has shown that varied levels of trace elements have marked effects on micro algae during cell division, and the addition of chealating agents like EDTA would increase the availability of trace elements to the algae. Gopinathan (1986) pointed out that no available culture medium is uniformly effective in all the cases, unless supplemented with either trace metals or vitamins according to the requirements of the algae. Further, he reported pointed out that *Chaetoceros calcitrans* showed some deviation from control growth in the absence of vitamin and trace elements

The study on transmission rate indicated that the elimination of one trace element at a time do not show much influence in the growth of the culture. In all these cases there is reduction in growth rate in comparison with control. It is clear that the vitamin elimination affects the growth of the culture along with the trace elements. Gopinathan (1986) pointed out that *Nitzshia closterium* has maximum growth in PM medium devoid of trace metals. Nowadays due to the industrial effluents the nutrient content were

increased in the seawater. The culture can grow in the expenditure of these nutrients present in the seawater itself. Srisudha (1989) and Rekha (2003) pointed out that the lower concentration of trace elements especially zinc has positive role in the growth of the micro algae like *Isochrysis galbana and Synechocystes salina.*

In the present study there was retardation in the cell concentration in all the trace elements and vitamins eliminated cultures on the exponential phase in comparison with control. From these results, it can be concluded that the elimination of vitamins affects the growth of the culture along with that of trace elements as in the case of transmission rate. From the cell concentration study it was quite obvious that the elimination of all the trace elements influence the growth of the culture adversely in all the cases.

Elimination of trace elements and vitamins showed variation in the chlorophyll *a* value from the control on the exponential day. All these indicated that there was difference in the chlorophyll *a* value in the absence of the vitamins and trace elements. The chlorophyll *a* study also gives a clear evidence for the addition of trace elements and vitamins in the seawater, for the better growth of the organism.

4.2 Effect on biochemical composition

4.2.1 Introduction

Micro algae specifically diatoms are the major source of food for all invertebrate larvae of marine organisms. The successful growth and development of cultured species depends to a great extent on the nature and biochemical content in the feed provided. According to Parson *et al.* (1961) the marine phytoplankton have very similar organic composition when grown under similar physical and chemical conditions, regardless of the size of the organism or the class to which it belongs. Biochemical composition of six micro algal groups was studied by Kaladharan *et al.* (1999) in laboratory conditions. The physical properties of the feed depend on the constituents; can also affect the value of food to an organism. Algal carbohydrates were considered to be nutritionally insignificant, but proteins at high levels, provide with high food values to algal diets (Utting 1986). The successful growth and development of the culture depends greatly upon the nutrients present in the culture medium. The salinity, temperature, pH etc of the medium also affects the growth and thereby the biochemical content of the cultured diatom. The protein component of the microalgal culture is the most important dietary nutrients. There have been very few attempts in the past to study

the nutritional requirements of the microalgae and the impact of these nutrients on the synthesis of various biochemical parameters likes protein and carbohydrate under laboratory conditions. In the present study the biochemical analysis was conducted to determine the optimum concentration of the media and the elimination of the trace elements on the biochemical constitution of the diatom *Chaetoceros calcitrans.*

Several attempts have been made to correlate the biochemical contents of phytoplankton to suitability as food for herbivores (Parsons *et al.,* 1961; Walne, 1970; Epifanio, 1979). Eventhough the biochemical composition of the algal species are qualitatively similar, there were some difference in quantity of some amino acids, proteins and total fatty acids. The biochemical constituents vary with different culture conditions. To avoid such problems, all cultures are maintained at the similar conditions and harvested at the same growth phase.

4.2.2 Materials and Methods

The diatom *Cheatoceros calcitrans* used for the biochemical study was taken from the stock culture maintained in the CMFRI laboratory. The micro algal species was grown in filtered seawater (Salinity 30 ppt) enriched with Conway medium (Walne, 1974) with additional silicate. The cultures were maintained at a

temperature of 25□1.8^{0}C under fluorescent light with frequent shaking. The same species was tried with different concentrations of medium at different days and also without the trace elements and vitamins as stated earlier. About 10 ml of sample from each flask were collected at an interval of five days for the biochemical study. The samples were centrifuged at the rpm 15000 for 20 minutes and the precipitates were collected.

Protein

The proteins from the samples were analyzed by using the method of Lowry *et al.* (1951).

Carbohydrate

The pellets of each sample were placed in a caped tube (15 ml) together with 5 ml of 5% TCA solution. The samples were heated for 5 hours, and then cooled and 0.2ml was pipette out and added 1 ml of 5% phenol and 5 ml of Concentrated H_2SO_4. Total carbohydrate was determined, according to Dubios *et al.* (1956) in a spectrophotometer of wavelength 490 nm.

Lipid

Method used for lipid estimation was Folch's followed by Phospho Vanillin method (1957). Centrifuging 5ml algal solution at 25,000 rpm for 20 minutes did lipid extraction and the pellet was taken. Then it was dissolved in 5ml of 2% chloroform and

methanol mixture. Now the extract is ready for lipid estimation. From the extract 0.2 ml is pipette out into a tube and 5ml of vanillin regent was added, mixed well and allowed to stand for 30 minutes and OD was taken at 520nm.

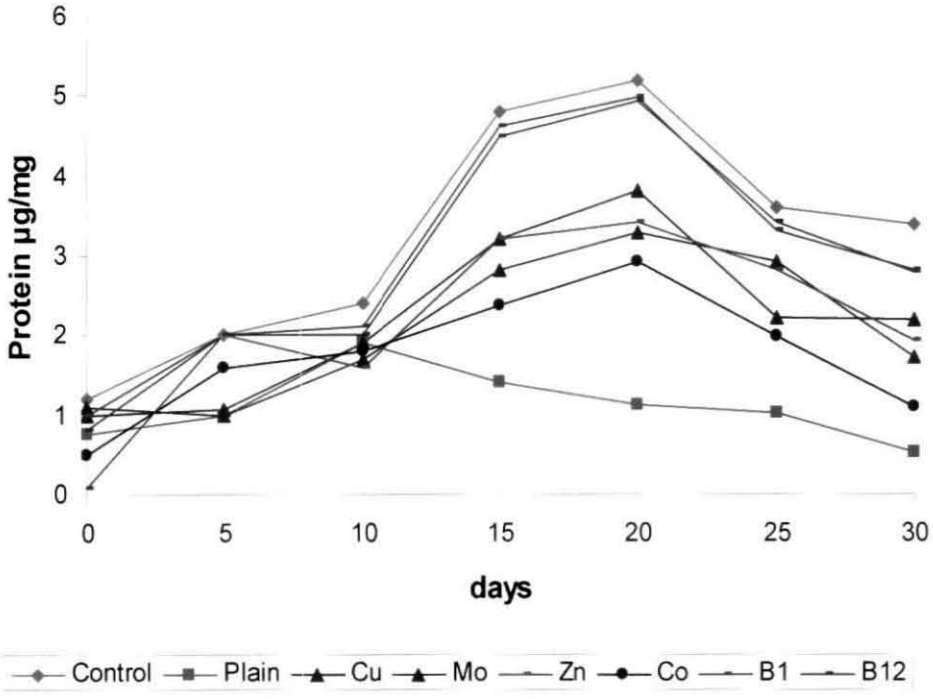
4.2.3 Results

Protein

For the control and treatment the protein value showed a gradual increase from the initial day onwards, and maximum value was observed on 20^{th} day followed by decrease on 25^{th} and 30^{th} day. But for the group in which no medium was added showed a growth only up to the 10^{th} day, there after declination. Protein values of all the treatment groups were lower than that of the control in every 5 days observation, indicating significant effect of elimination of trace element on protein. The protein values of vitamin eliminated cultures were almost similar to that of the control without much significant difference between them. At the same time the trace element eliminated cultures showed reduction in protein value from the control. On the exponential day also B_1 and B_{12} showed almost similar value to the control. Cu showed a protein value which was 32.5% less than the control, while that for Mo, Zn and Co was 36.9, 34.4 and 43.8% respectively. Not much

variation was noticed between the treatment groups except the plain (Fig. 4.4).

Fig. 4.4 *Protein content on nutrient elimination*



The ANOVA indicates that there was significant effect for the elimination of trace elements and vitamins and the age of culture on the protein value. It was also observed from the Duncan's analysis that the culture without the medium showed significantly ($P>0.01$) lower values of protein over the control. Among the treatment groups maximum protein value was for the vitamin B_{12} elimination followed by the vitamin B_1, and other treatments formed homogenous group. It was also clear that the significantly higher values were observed on 20^{th} day than others (Table 4.4).

Table 4.4 DMRT for protein content on nutrient elimination

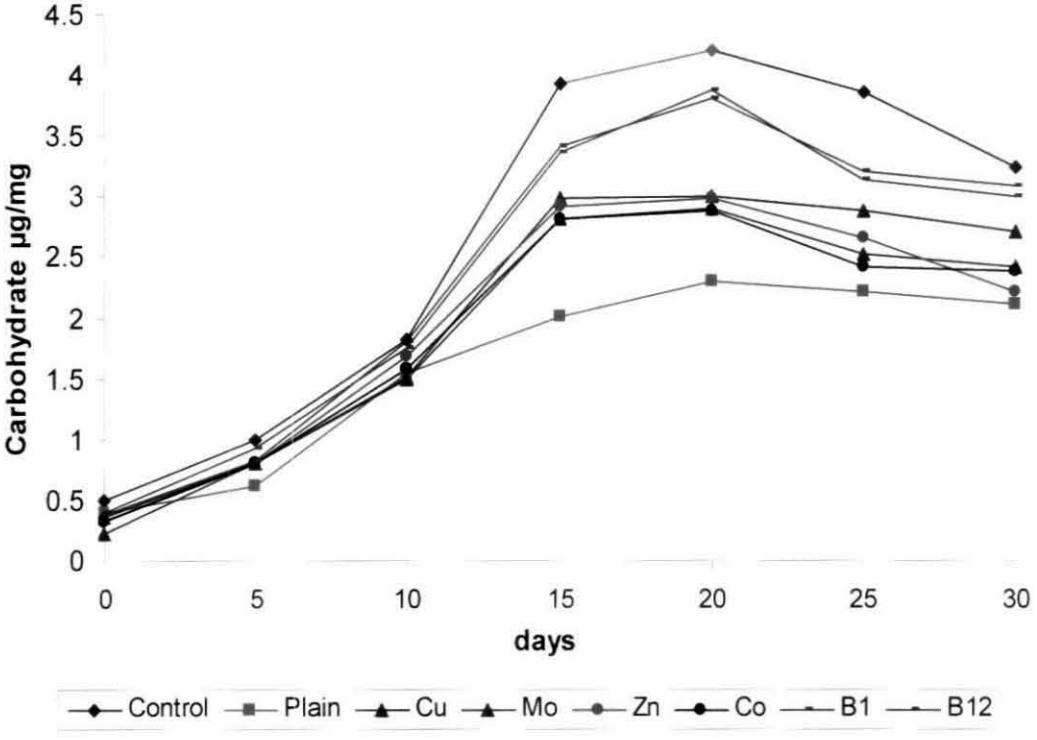
Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	141.508	13	10.885	21.664	.000
Intercept	560.181	1	560.181	1114.909	.000
Day	80.096	6	13.349	26.569	.000
Treatment	61.412	7	8.773	17.461	.000
Error	49.240	98	.502		

Significant at (P<0.01) level.

Carbohydrate

Upto the 10th day almost similar carbohydrate values were observed. But on 5th day the control showed maximum value of 3.94µg/mg followed by the vitamin B_1 and B_{12}, 3.36 and 3.41µg/mg respectively with a decrease of 14.7% and 13.45%. All the others showed more or less same value 2.99, 2.82, 2.92 and 2.81 respectively for Cu, Mo, Zn and Co. Here control showed an increase of 24.11, 28.4, 25.8 and 28.61% Cu, Mo, Zn and Co respectively. On the exponential phase, the vitamin B_1 and B_{12} eliminated culture showed values nearest to the control showing a decrease of about 4.4%, all the others forms a group with about a decrease of 36.5-43.8%. In the declination phase also less deviation was observed for vitamin B_1 and B_{12} eliminated culture, others showed lower values of carbohydrate than the control (Fig. 4.5).

Fig. 4.5 *Carbohydrate content on nutrient elimination*



The two way ANOVA indicated that the carbohydrate value was significantly affected by the elimination of the trace elements and the age of culture. From the DMRT it was clear that the control has significantly ($P<0.01$) higher values than others. Among the treatment groups vitamin B_1 and B_{12} eliminated formed one group and trace element eliminated treatments formed another homogenous group indicating that the elimination of trace elements will adversely affect the culture. It was also clear that the carbohydrate value on the initial day was least significant than that on 20^{th} day (Table 4.5).

Table 4.5 DMRT for carbohydrate on nutrient elimination

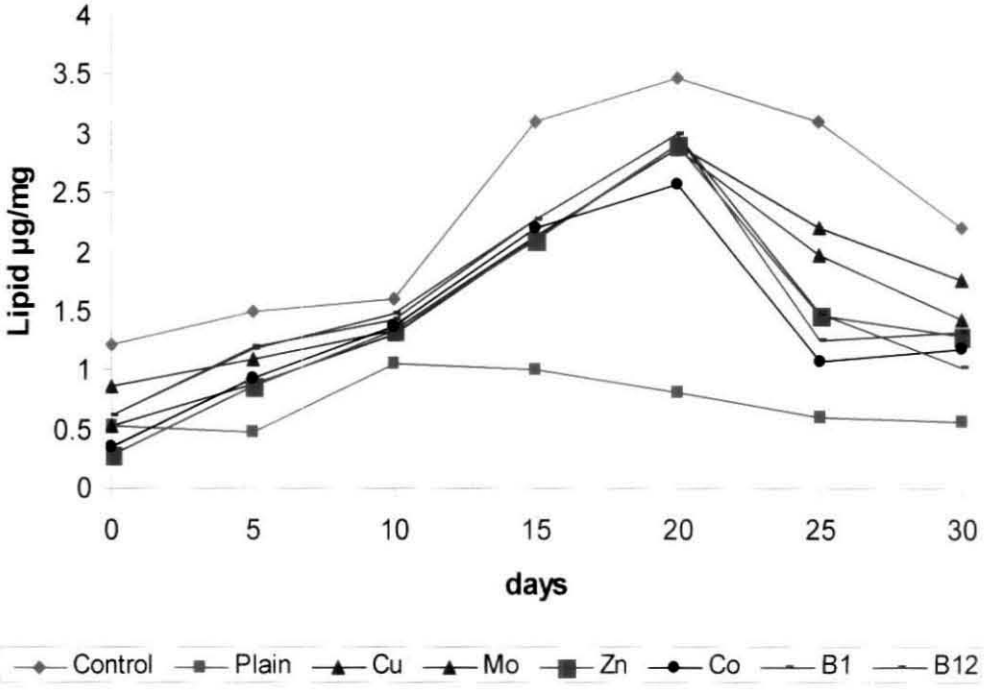
Source	Type III Sum of Squares	Df	Mean Square	F	P
Corrected Model	134.498	13	10.346	120.072	.000
Intercept	472.239	1	472.239	5480.637	.000
Treatment	13.958	7	1.994	23.142	.000
Days	120.540	6	20.090	233.158	.000
Error	8.444	98	8.617E-02		

Significant at ($p<0.01$) level.

Lipid

Lipid values of all the treatment were almost same upto the 15th day without much deviation between them, but there was a decrease from the control. The lipid value for control on 15th day was 3.1µg/mg, for Cu it was 2.11, while it was 2.6, 2.9, 2.4, 2.28 and 2.28 µg/mg respectively for Mo, Zn, Co, vitamin B_1 and B_{12}. On the 20th day least lipid value was observed for Cu, all others are in the same rate, 3.48 for control, 2.97, 2.88, 2.81, 2.58, 2.99 and 2.99µg/mg for Cu, Mo, Zn, Co, vitamin B_1 and B_{12} respectively. In the declination phase the maximum declination was for Co followed by Zn, Vit. B_1, Mo and Cu respectively (Fig 4.6).

Fig. 4.6 *Lipid content on nutrient elimination*



The two way ANOVA indicates that the lipid value was significantly affected by the elimination of the trace elements and the age of culture. From the DMRT it was clear that the control has significantly ($P<0.01$) higher values than others. Among the treatment groups Mo has maximum lipid value followed by vitamin B_1, vitamin B_{12}, Cu, Zn and Co at the same time all of them forming homogenous group. It was also clear that the lipid value on the initial day was least significant than that on 20^{th} day (Table 4.6).

Table 4.6 DMRT for lipid on nutrient elimination

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	70.498	13	5.423	40.193	.000
Intercept	271.005	1	271.005	2008.626	.000
Treatment	18.958	7	2.708	20.073	.000
Days	51.540	6	8.590	63.667	.000
Error	13.222	98	.135		

4.2.4 Discussion

The potentiality of diatoms as source of food/feed has been further established by the extensive researches carried out during the past two decades. The economic utilization of diatoms necessitates the development of techniques for axenic culturing of these organisms on a large scale. Though many media has been devised for culturing different microalage, in the opinion of Venkataraman (1960 a), that no single medium can be said as the best one. From the studies with different algal species, Gopinathan (1986) also arrived the same conclusion. Since the nutritional requirements of diatoms vary with species, the successful and long term culturing of any species demands a thorough understanding of its nutritional requirements which can be studied only under controlled laboratory conditions.

Analysis of natural crops and cells in pure culture have been performed on organisms grown under a variety of conditions and using different techniques for the estimation of their composition (Vinagradov, 1953, Strickland, 1960). Diatoms are known to contain carbohydrate associated with silica of the frustules (Fogg, 1953). According to Parson *et al.* (1961) *Skeletonema* contain less carbohydrate than glucose. The governing factor in the composition of algae appears to be the physical and chemical environment in which it is grown (Spoher and Milner, 1949). Similar conclusion was reached by Ketchum and Redfield, (1949) from a less exclusive study of five Chlorophyceae and one diatom.

In the present investigation, an attempt has made to assess the role of trace elements like copper, ammonium molybdate, cobalt, zinc and vitamin B_1 and B_{12} each in Walne's medium of *Cheatocros calcitrans* culture. The study also puts an endeavor to evaluate the optimum concentration of these nutrients that would provide maximum output of the culture. The various biochemical parameters observed include protein, carbohydrate and lipid.

Cobalt is a trace metal which behaves as a nutrient in some marine phytoplankton species. Although cobalt concentrations in the open ocean are typically at least an order of magnitude less than those of zinc, in some regions of the ocean where zinc is extremely

depleted, cobalt concentrations approach those of zinc. Laboratory cultures of marine phytoplankton demonstrate that cobalt additions to culture media can alleviate zinc limitation of growth. According to Sunda and Huntsman (1995), Co and Cd can substitute Zn in some phytoplankton species. In trace metal eliminated treatments, least value of carbohydrate was observed. Least difference was noticed for B_1 and B_{12} respectively. All these indicate that trace elements elimination plays negative role in the growth of the culture.

Lipid value in trace elements eliminated cultures showed a reduction. It can be concluded that elimination of each trace metal and vitamin has its own effect on the lipid value. As in the case of difference in the transmittance, cell concentration and chlorophyll *a* content by the elimination of trace elements from the control, biochemical studies showed that there is reduction in the protein, lipid and carbohydrate content of the culture. This indicates that for the body building of the system the trace elements and vitamins are necessary.

Chapter 5

5. Correlation of diatom population with pelagic fish landings along the south west coast of India

5.1 Introduction

Certain diatoms are useful to identify the water masses and currents in various places due to their distribution and their relationship with climate and physical properties of water. They are also useful as indicators to forecast the availability of particular organisms especially fishery by the nature of their abundance and of the particular species. In European waters only the Pilchard or sardine has been shown to feed on diatoms, while in Pacific, anchovy is a diatom feeder. The blooming of the diatom *Fragilaria oceanica* indicates the abundance of oil sardine in that area. While Prasad (1959) reported that in east coast *Hemidiscus hardmanianus* indicates the abundance of choodai fishery. As the primary tropic level of the food pyramid in any aquatic system, and also as source of food of plankton-eating fish, it is important to know how we can manipulate the plankton density especially diatoms, qualitatively and quantitatively, in the fish production.

Practically no work has been carried out about correlation aspect between the diatom and the pelagic fish landings along the south west coast of India. The present study is an attempt to know

the role of diatoms as live feed of pelagic fishes such as oil sardine, mackerel and anchovies along the south west coast of India.

5.2 Material and Methods

Three stations were selected along the south west coast of India namely Thalassery, Cochin and Vizhinjam, and monthly samples were collected for estimating the diatom population. An attempt to correlate the diatom population with fish catch along the area was also done by collecting the data of pelagic fish catch during the year 2001-2002 obtained from Fishery Resources Assessment Division of CMFRI. From the data, the fish landings of diatom feeders such as mackerel, sardine, and anchovies along the three stations were taken, average value was calculated and correlation study was conducted in the SPSS followed by t-test and the graph was plotted between the diatom population and the fish landings along the above said stations.

5.3 Results

It was found that along the Thalassery and Vizhinjam regions, there was a positive correlation between the pelagic fish landings and the total diatom population (0.01%). But along Cochin, less significant correlation was noticed between the diatom population and the pelagic fish landings (0.05%). Figs. 5.1, 5.2 and 5.3 represents the correlation picture of the Thalassery, Cochin and Vizhinjam areas respectively.

Figure 5.1 ***Diatoms and pelagic fish landings-Thalassery***

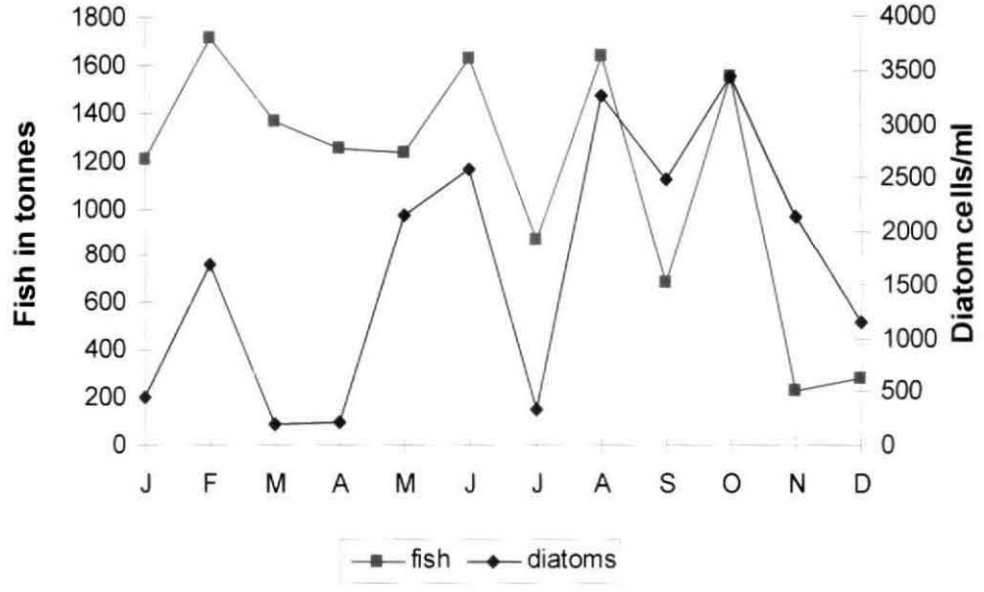


Fig.5.2 ***Diatoms and pelagic fish landings -Cochin***

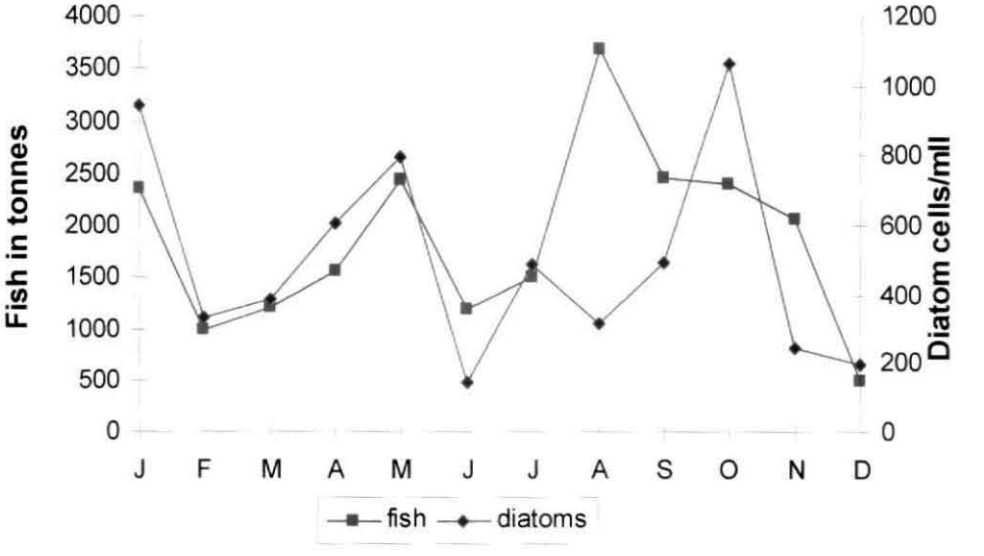
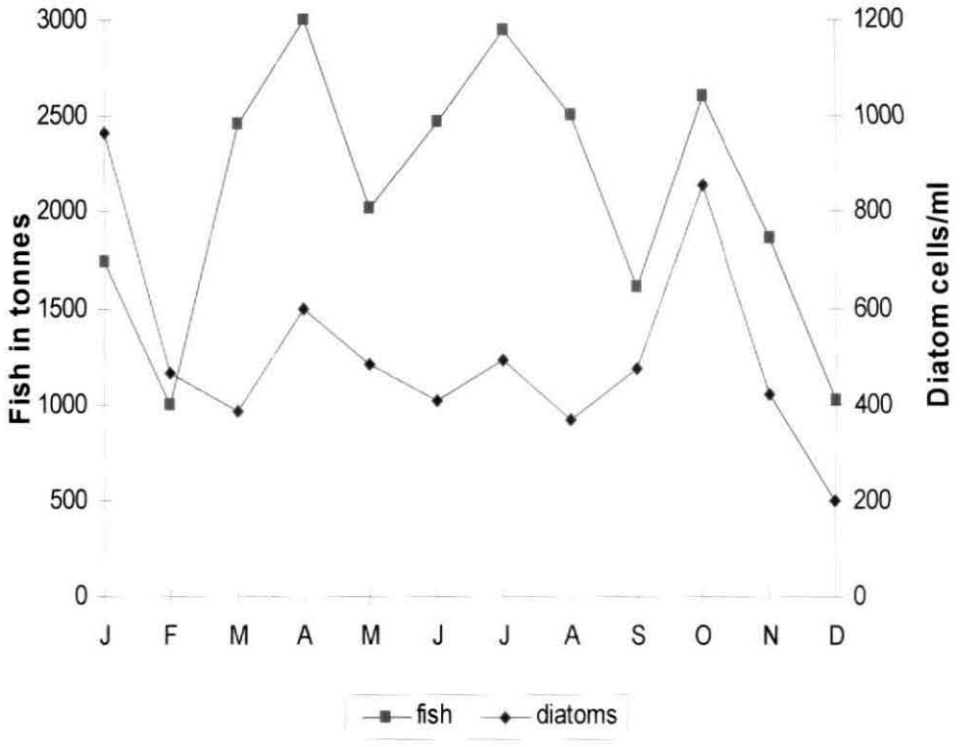


Figure 5.3 ***Diatoms and pelagic fish landings-Vizhinjam***



5.4 Discussion

The magnitude of the production of phytoplankton in different waters has been estimated by various methods by several authors and generally it has been found to be several times that of commercial landings of fish. Several methods have been adopted in arriving at the estimation of production of phytoplankton and organic matter such as oxygen production during photosynthesis, photosynthetic pigments and utilization of carbon-di-oxide (^{14}C technique) and even nutrients of the seawater. For the English

Channel, Cooper (1933) has calculated the intensity of phytoplankton production based on the consumption of CO_2 and nutrients and production of oxygen. Later Subrahmanyan (1959) calculated the production of phytoplankton on the west coast of India based on the pigment analysis by Harvey units and indicated that the total landings of the commercial fish represents only a very small fraction of the total production of phytoplankton and pointed out that there is vast scope for increased exploitation of the potential resources with positive results in the west coast of India. Further, Prasad *et al.* (1970) estimated the organic production of the Indian Ocean (51 m. sq. Km) was 3.9x 10^9 tonnes of carbon which amounts as $1/5^{th}$ of world oceanic production.

In recent years, various projections of potential resources have been made from the estimates of primary production. The optimum yield from organic production varies from 0.3 to 0.4% (in terms of carbon-10% of the wet weight or 50% of protein) in the inshore and coastal areas. Gopinathan (1981) has accounted 0.2% of conversion efficiency from primary to tertiary production and reported that about 283 m. tonnes of carbon are produced annually from the EEZ of India and potential harvestable resources will be about 5.5 million tonnes from these areas.

Correlation study revealed that the production of diatoms with the pelagic fish landings in Indian seas and elsewhere are scanty. Prasad *et al.* (1970) have studied the regional and seasonable variability and magnitude of primary production in the inshore environment of the west coast of India in relation to the potential fishery resources. A correlation study of the total fish landings and potential productivity of the waters along the mud bank of Alleppy, west coast of India, was studied by Nair and Gopinathan (1977) and indicated a positive correlation. Subrahmanyan (1959) observed that the bloom of *Fragilaria oceanica* could be used as an indicator of the abundance of oil sardine and the sequence of appearance and disappearance of fishes were related to the water movements and diatom blooms. Verlancar (1978) studied bloom of the blue green algae *Trichodesmium* at several places along the south west coast of India and it was seen that there were good catches of oil sardine and mackerel throughout the west coast. Of course, pelagic fishes like sardine, mackerel and anchovies try to avoid certain dense areas of algal blooms temporarily. From these accounts, it can be incurred that some of the phytoplankton blooms occurring along the Indian coast have some indirect short term influences on pelagic fishes.

In the present investigations, although the potential resources estimation has not been attempted, a correlation study has been under taken between the diatom population of the selected areas of the south west coast of India and pelagic fish landings of these areas. It was found that along the Thalassery and Vizhinjam area, there was a clear positive correlation between the diatom population and the pelagic fish landings while at Cochin waters less significant correlation noticed between these two variables. This study also confirms the unique role of diatoms as the basic food of the pelagic fishes of the south west coast of India.

Conclusion

The present investigation on **"Studies on diatoms along the south west coast of India in relation to the hydrological parameters"** is an attempt to study the influence of various hydrobiological parameters on the distribution and ecology of diatoms along these areas and to determine the effect of one of the important hydrological parameter, especially nutrients on the diatom *Chaetoceros calcitrans* under laboratory conditions.

Another study was carried out to assess the optimum concentration of Walne's culture medium for the growth of the diatom *Chaetoceros calcitrans*, by providing various concentration of the medium ranging from 25-100% at different days. The effect of elimination of trace elements and vitamins on *Chaetoceros calcitrans* under laboratory conditions was also studied by eliminating the trace elements and vitamins from the culture medium. Above all, a correlation study between the diatoms of the above said areas of south west coast of India with the pelagic fish landings (sardine, mackerel and anchovies) of the same area was also attempted.

The salient and significant features based on the results of the study are as follows:

- Inshore and nearshore areas showed variation in different productivity parameters from season to season and within season.
- Along nearshore area, the monsoon season is with high richness and abundance of diatom while that for the Inshore area is post monsoon season.
- Factors contributing the diatom population are different in each station.
- Addition of the medium on the fifth day of inoculation in *Chaetoceros calcitrans* culture is enough for the proper growth of the culture under controlled conditions.
- Elimination of trace elements and vitamins affect significantly the transmission, cell concentration, chlorophyll *a* and the biochemical contents, of the diatom culture.
- Positive correlation observed between the diatom concentration and the pelagic fish landings along the selected areas of south west coast of India.

Summary

Summary

The distribution and abundance of diatoms along the South west coast of India in relation to the hydrological parameters was studied by selecting three stations namely, Thalassery, Cochin and Vizhinjam. The present study has been carried out with a view to study the diatoms of selected areas along the south west coast of India in relation to the hydrographic factors. Qualitative and quantitative studies were made along the nearshore and inshore areas following the standard procedures. Regression analysis was also conducted to study the various factors contributing the growth of diatoms along the near shore and inshore areas of the three selected stations. It was found that the hydrological parameters showed fluctuation from season to season and within season. Diatoms along the nearshore areas showed abundance and dominance during the monsoon season followed by the post monsoon season, while that along the inshore areas are having the highest dominance and abundance during post monsoon followed by monsoon season. The regression analysis indicates that the diatom population was contributed by different factors at different stations indicating that they are all independent.

There have been very few attempts in the past to study the nutritional requirements of micro algae under laboratory conditions. In this account an attempt was made to study the optimum concentration of culture media required on suitable period of culture of *Chaetoceros calcitrans* under controlled conditions so as to achieve a viable and economical method of culture. The main objective of the study was primarily to determine the effect of hydrological parameters especially nutrients which was supplied externally to the seawater while culturing the diatom under laboratory conditions, and to determine its optimum concentration for ideal growth of the culture, in such a condition of increased rate of pollution due to the industrialization and effluents. The cell count, percentage of transmittance and the chlorophyll *a* values are significantly higher for the group in which the medium was provided on the initial day. Almost similar values were also noted for the 5^{th} day's enrichment, indicating that the culture can grow upon the nutrients present in the seawater up to the 5^{th} day and enrichment is needed only on 5^{th} day.

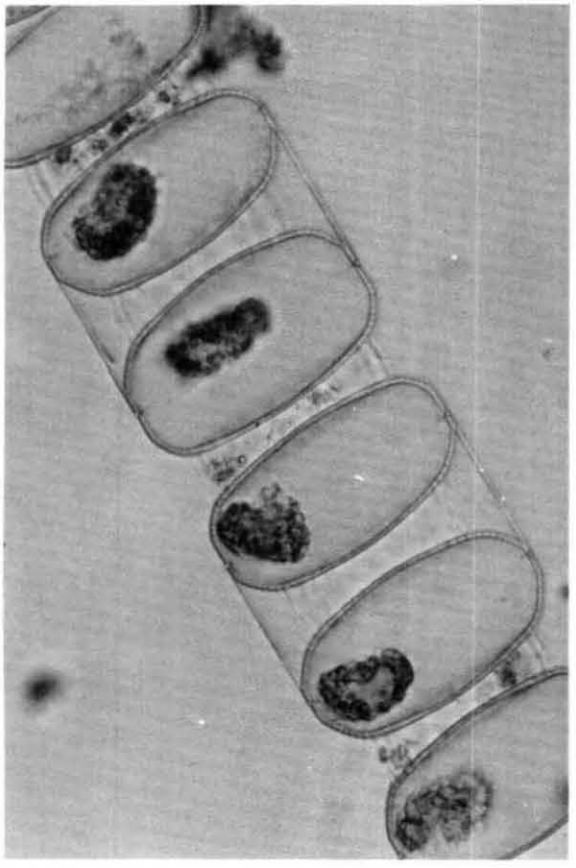
Another experiment was also conducted to study the effect of elimination of trace elements and vitamins on the growth and biochemical contents of the culture of *Chaetoceros calcitrans* under laboratory conditions. The control showed significantly

higher cell concentration rate and higher chlorophyll *a* content and lower transmission rate than the others indicate higher growth. Between the treatment groups there was not much variation in the growth pattern, indicating that the elimination of the trace elements and vitamins affect adversely the growth of *Chaetoceros* culture. The control indicated significantly higher values of protein, carbohydrate and lipid than the treatment groups, indicating that the elimination of the trace elements will affect the biochemical content of the culture. The values of protein and carbohydrate for vitamin B_1 and B_{12} eliminated medium was similar to that of the control showing that the elimination of vitamins did not affect much on the protein and carbohydrate content of the culture.

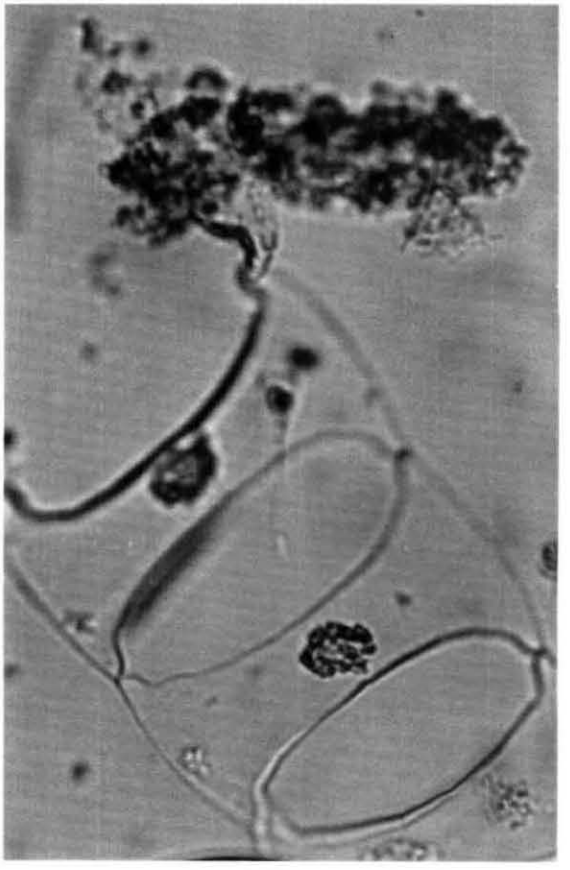
Also an attempt has been made to study the possible correlation of diatom abundance in the south west coast of India with the landings of the pelagic fishery resources, especially sardine, mackerel and anchovy, since these fishes are purely diatom feeders. Positive correlation was found between the diatoms and the pelagic fish landings of the Thalassery and Vizhinjam area while at Cochin there was less significant correlation between the two variables.

Common diatoms of south west coast of India

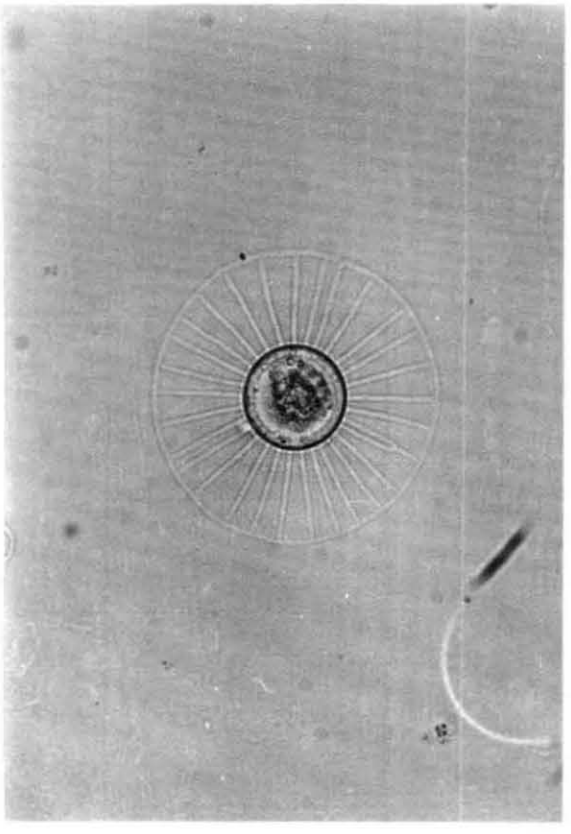
Plate 2. Centrales



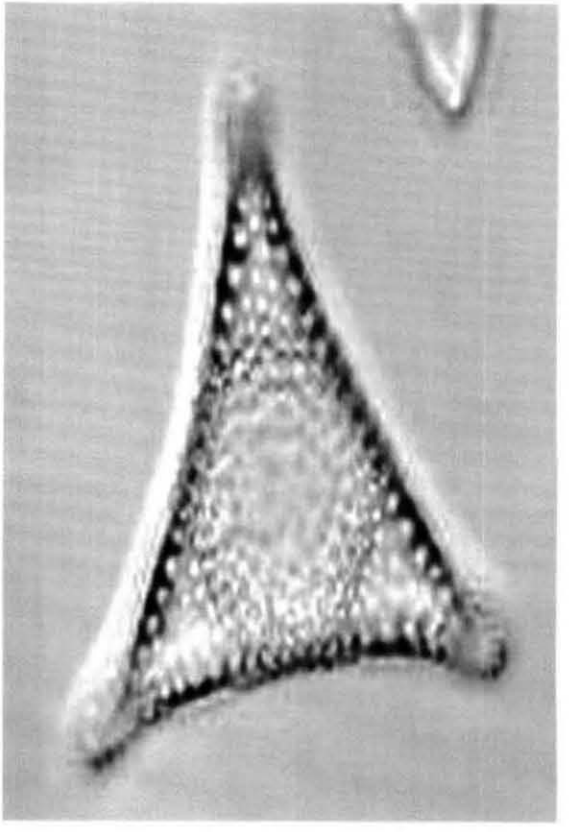
Skeletonema costatum



Climacodium frauenfeldianum

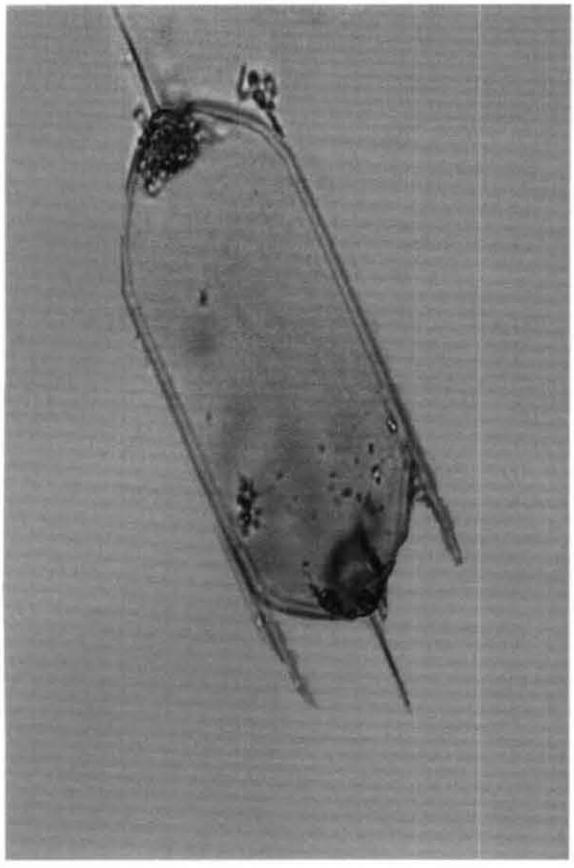


Planktoniella sol

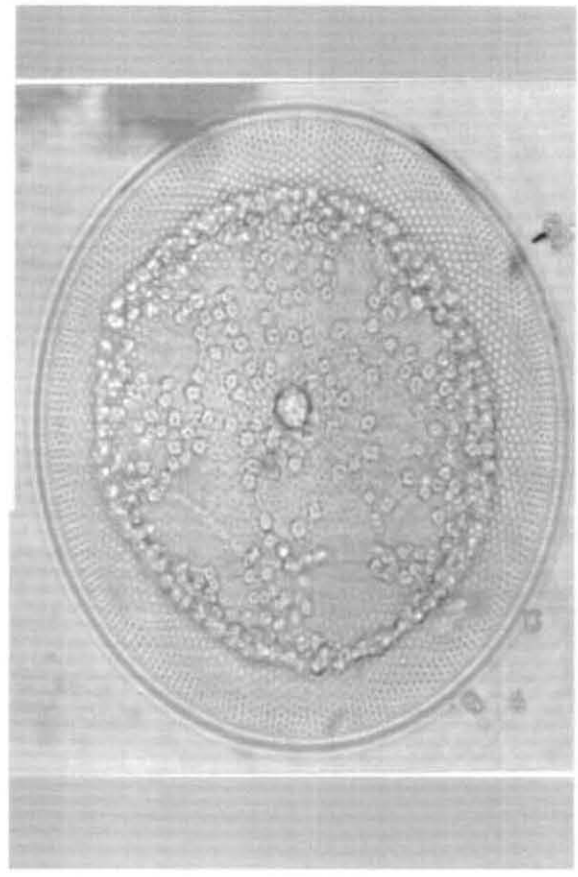


Triceratium reticulatum

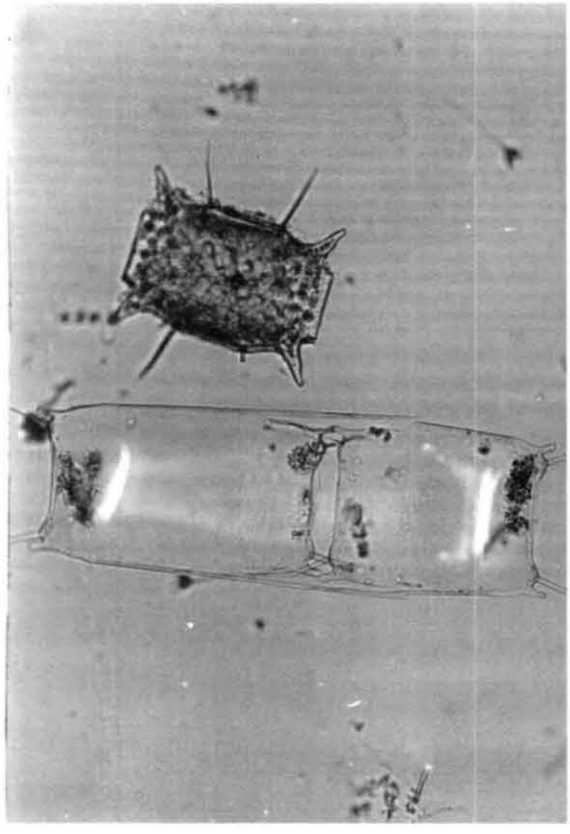
Plate 3. Centrales



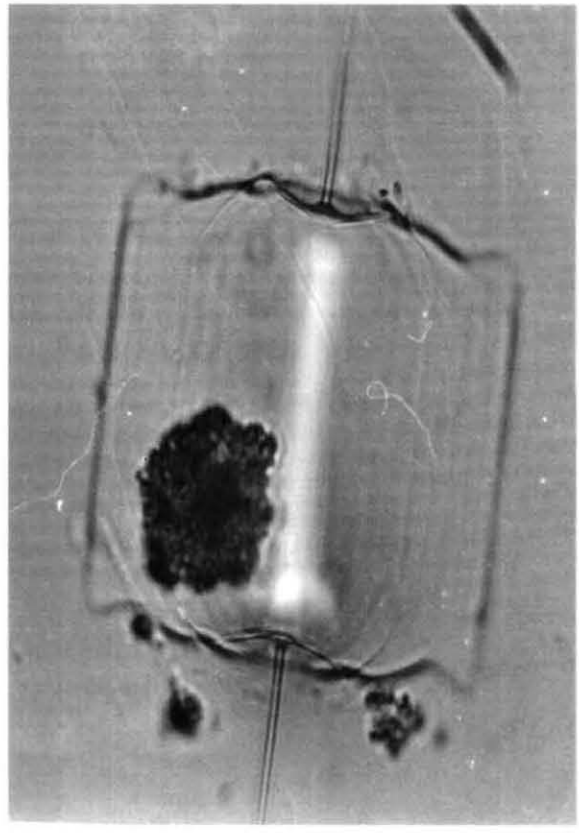
Ditylum **sp**



Coscinodiscus marginatus

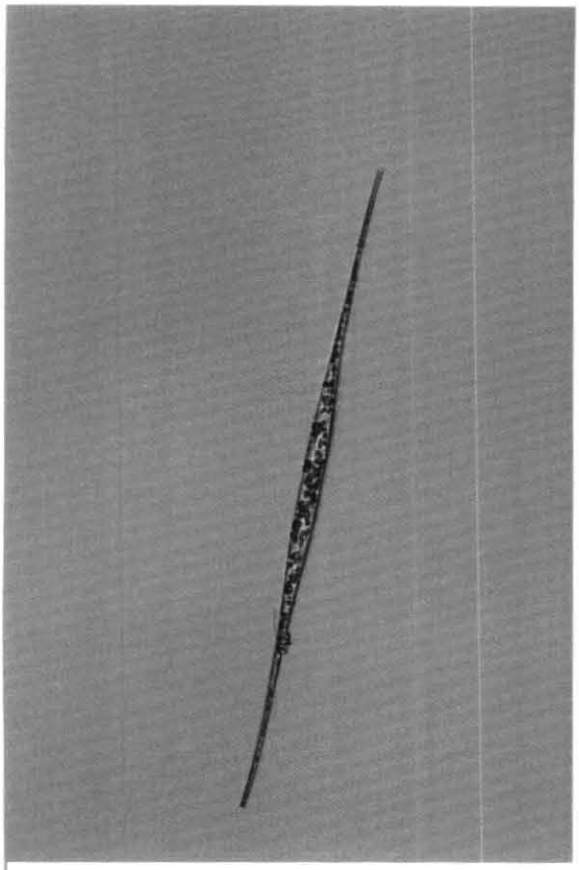


Biddulphia mobilensis & B. sinensis

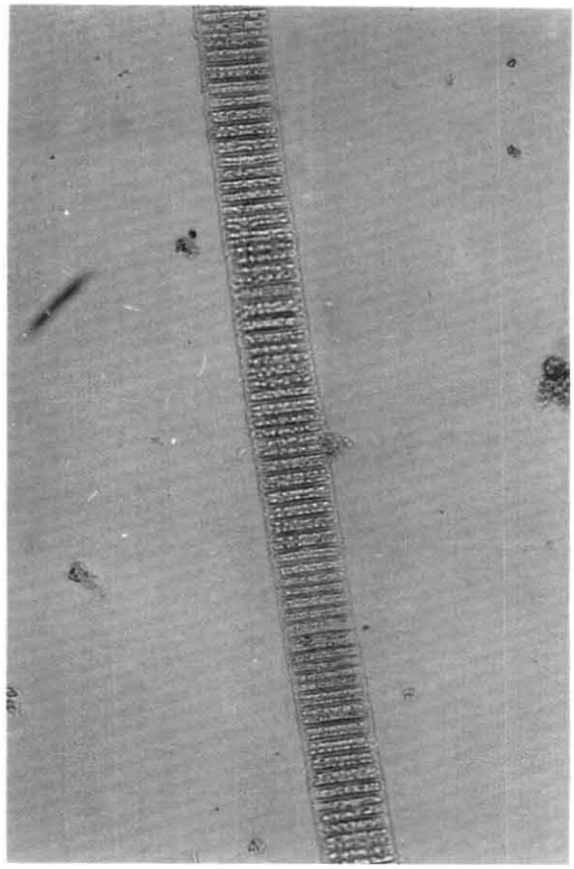


Ditylum brightwellii

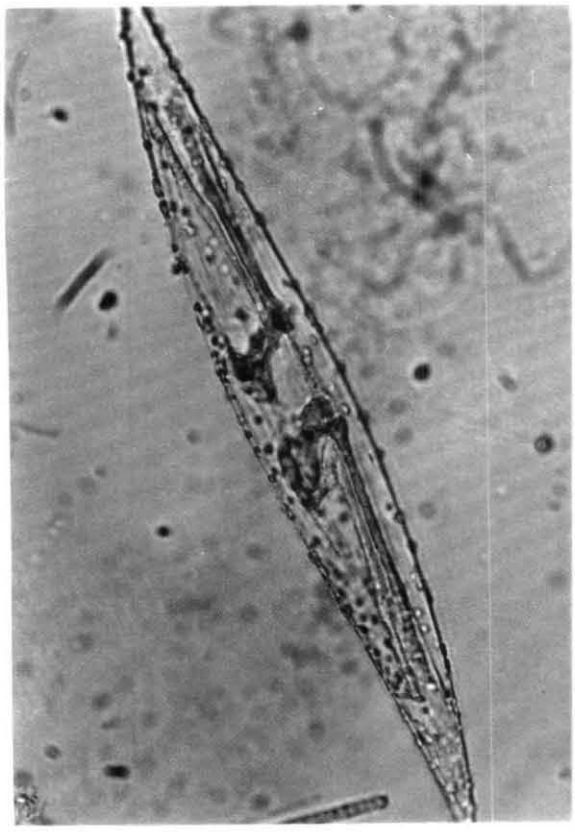
Plate 4. Pennales



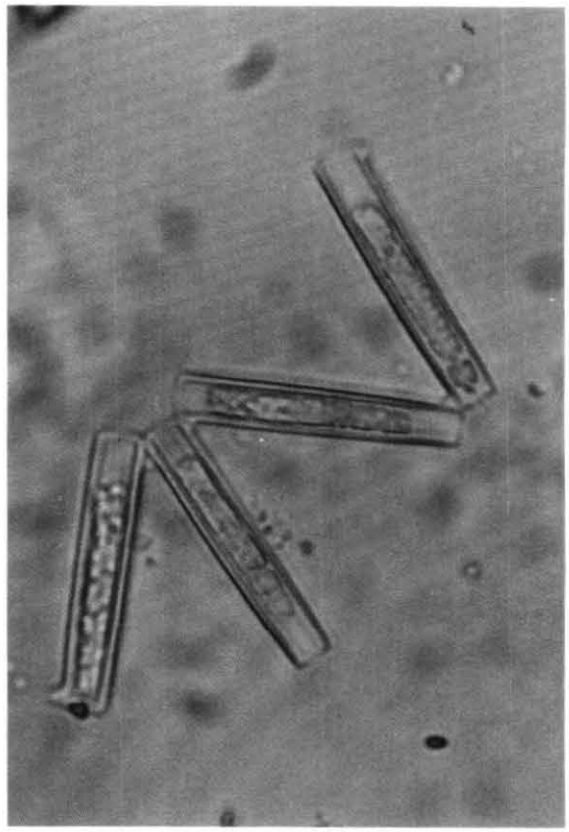
Nitzschia longissima



Fragilaria oceanica

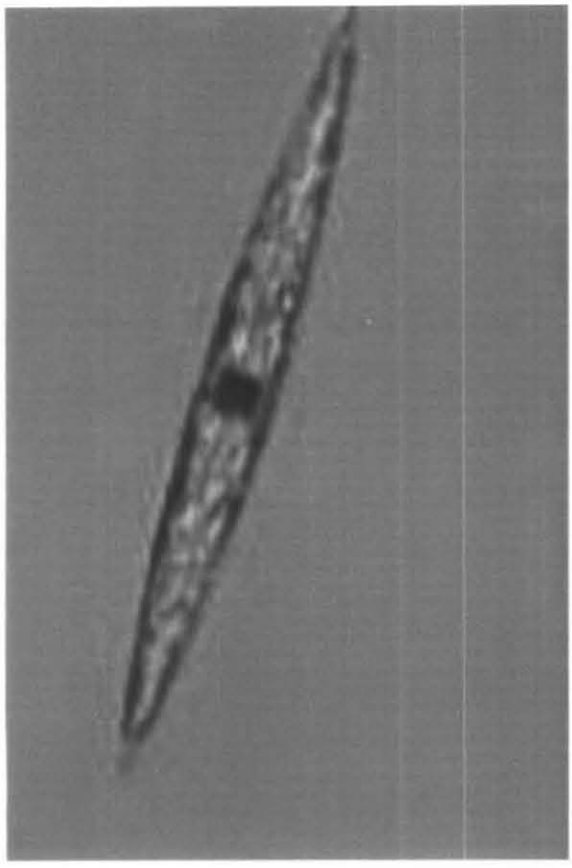


Pleurosigma elongatum

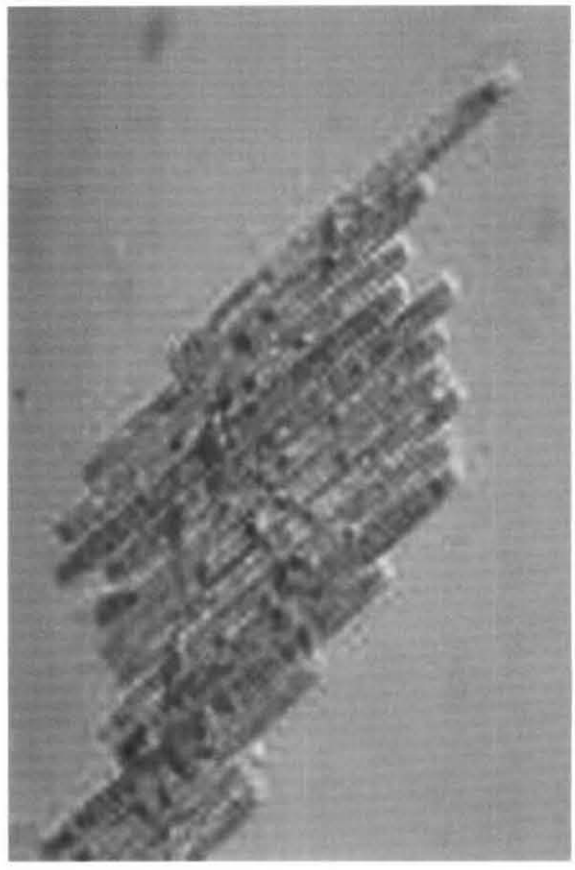


Thalassionema nitzschiodes

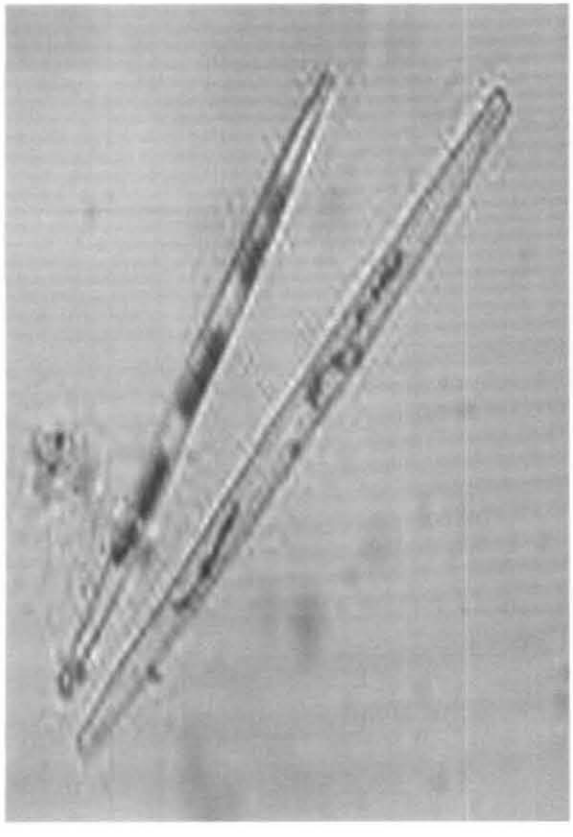
Plate 5. Pennales



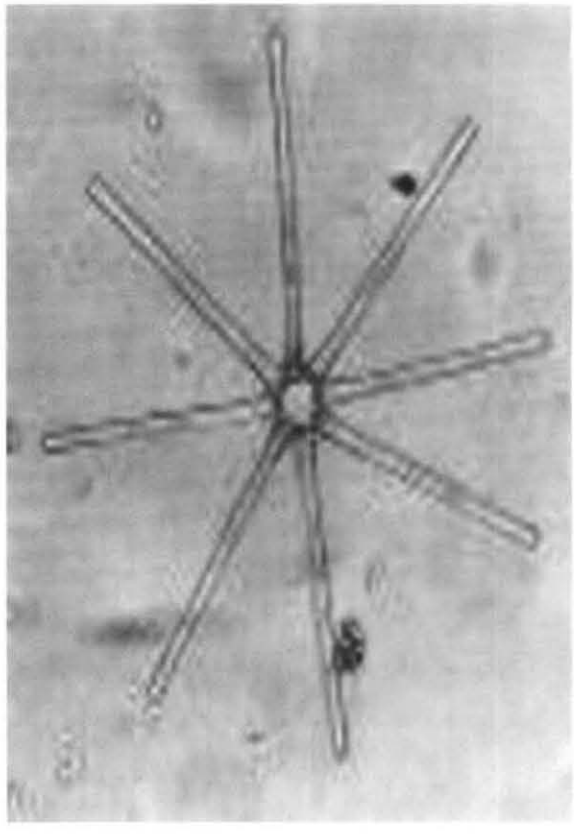
Navicula longa



Bacillarian paradoxa



Synedra formosa



Asterionella japonica

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